Proteomic analysis of the *Candida albicans* secretome and its antigenic properties in the human host

Dissertation

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All under heaven realizing beauty as beauty, wickedness already.
All realizing goodness as goodness, no goodness already.
Hence existence and nothing give birth to one another,
Difficult and easy become one another,
Long and short form one another,
High and low incline one another,
Sound and tone blend with one another,
Front and back follow one another.
That is forever.

-- Tao Te Ching, Laozi

Like that, *Candida albicans* is a friend of humans, as well as an enemy!

Summary

Candida albicans is a common opportunistic fungal pathogen, which normally resides on mucosal surfaces of healthy individuals as a harmless commensal. However, in immunocompromised and critically ill patients, the inability to control colonizing *C. albicans* on mucosal surfaces can lead to superficial infections and even disseminated candidemia. This severe *Candida albicans* infection is usually associated with a high mortality of up to 46%-75% in infected populations. So far the diagnosis of *Candida* infections remains difficult.

During infection, *C. albicans* undergoes a yeast-to-hypha transition and secretes numerous proteins for invasion of host tissues and modulation of the immune response. It is assumed that mainly the hyphal form is associated with the invasive phenotype of *C. albicans* and transition from colonization to infection is accompanied with a yeast-to-hypha transition. Hence, it is particularly interesting to investigate the dynamic changes of extracellular proteins during the yeast-to-hypha transition. Still little is known about the interplay of *C. albicans* extracellular proteins with the host humoral immunity. Investigation of the extracellular protein antigens of *C. albicans* could therefore give new insights into this question and help search for novel biomarker candidates for diagnosis of *C. albicans* infections.

Here, we combined 2D gel- and LC-MS/MS-based approaches for characterization of the dynamic changes of the *C. albicans* secretome during yeast-to-hypha transition. In order to meet the large demand of protein amount for 2D-GE analysis, we set up a workflow for the cultivation of *C. albicans* yeast and hyphal cells in a batch-fermenter. The combination of both techniques led to a more comprehensive *C. albicans* secretome map. A total of 101 extracellular yeast proteins and 410 extracellular hyphal proteins were identified. Among them, 65 proteins were shared by both cell morphologies, while 36 proteins were exclusively detected in yeast and 345 only in hyphal cells. Both *C. albicans* yeast and hyphal secretomes mainly consist of cell wall-associated proteins, proteins in response to environmental stimuli, secreted proteases, lipases and adhesins and carbohydrate metabolism-associated proteins. Besides extracellular proteins, intracellularly processed peptides in the supernatant of *Candida* hyphal culture were also investigated. Ece1p turned

out to be the most abundant intracellular processed protein in the culture supernatant. Intriguingly, the Ece1p-III peptide was identified as the first fungal cytolytic peptide toxin. It can damage epithelial membranes directly and trigger a danger response pathway to activate epithelial immunity. In addition, the fasciclin domain-containing protein CaO19.3004, which has a domain with repeated KR sites similar to Ece1p and a conserved cell adhesion domain (Fas-domain), was found to be another abundant, proteolytically processed protein.

Further, we focused on the serological responses of the host to *C. albicans* extracellular proteins. In mice systemically infected with *C. albicans*, the serological responses to the secreted proteins of *C. albicans* yeast cells were less pronounced and diverse in comparison to humans. An in-depth investigation of the serological response of candidemia patients to extracellular proteins of *C. albicans* was conducted by a 2D-immunoblotting approach combined with mass spectrometry for protein identification. Based on the screening of sera from candidemia patients and three control groups (systemic inflammatory response syndrome, bacteremia patients and healthy controls) a core set of 20 immunodominant anti-*C. albicans* extracellular protein antibodies was identified, seven of which represent potential diagnostic markers for candidemia (Xog1p, Rot2p, Eno1p, Met6p, Tsa1p, Tpi1p and Prx1p). Intriguingly, some secreted, strongly glycosylated protein antigens showed high cross-reactivity with sera from non-candidemia control groups. Enzymatic deglycosylation of hyphal secreted proteins significantly impaired sera antibody recognition. In addition, deglycosylation of the recombinantly produced, secreted aspartyl protease Sap6p confirmed a significant contribution of glycan epitopes to the recognition of Sap6p by serum antibodies of patients.

In summary, this study provides new insights into the serological response of candidemia patients to extracellular proteins of *C. albicans* yeast and hyphal cells. Seven *C. albicans* extracellular antigens were identified as promising diagnostic biomarker candidates for the diagnosis and prognosis of candidemia. In particularly, the mechanism underlying the high cross-reactivities of *C. albicans* extracellular antigens was elucidated. As shown here, glycosylation of *C. albicans* extracellular proteins enhanced the immunogenicity of glycopeptide epitopes and also led to relatively unspecific binding of antibodies, resulting in cross-reactivity of *C. albicans* protein antigens.

Kurzzusammenfassung

Candida albicans ist ein häufig vorkommender, opportunistisch human pathogener Hefepilz bei Menschen. Er besiedelt normalerweise als kommensaler Organismus der mikrobiellen Flora die Schleimhäute vieler gesunden Menschen. Geht jedoch die immunologische Kontrolle über die Besiedlung der Mucosa durch *C. albicans* in immunsupprimierten oder schwerkrankenden Patienten verloren, kann *C. albicans* Oberflächeninfektionen, aber auch lebensbedrohliche, systemische Infektionen verursachen. Systemische Infektion gehen meist mit hoher Sterblichkeit von 46% bis 75% in infizierten Populationen einher. Bisher ist die Diagnose einer *C. albicans*-Infektion schwierig und häufig nicht eindeutig.

Während der Infektion ändert *C. albicans* seine Morphologie von der Hefe- in die Hyphenform und scheidet zahlreiche Proteine zur Invasion des Wirtsgewebes und zur Modulation der Immunreaktion aus. Noch wenig ist über die Wechselwirkung zwischen extrazellulären Proteinen von *C. albicans* und der humoralen Immunantwort des Wirts bekannt. Deshalb war es besonders interessant, die extrazellulären Proteinantigene von *C. albicans* zu untersuchen und nach neuen Biomarkerkandidaten für die Diagnose von *Candida*-Infektionen zu suchen.

In dieser Arbeit wurden 2D Gel- und LC-MS/MS-basierte proteomische Methoden verwendet, um die dynamische Veränderung des Sekretoms von *C. albicans* während der morphologischen Umwandlung von Hefe- zu Hyphenzellen zu untersuchen. Dies führte zu einer umfassenden Charakterisierung des *C. albicans* Sekretoms. Insgesamt wurden 101 extrazelluläre Hefeproteine und 410 extrazelluläre Hyphenproteine identifiziert. Unter ihnen befanden sich 65 Proteine, die in beiden Zellmorphologien gefunden wurden, während 36 Proteine nur in Hefe- und 345 ausschließlich in Hyphezellen detektiert wurden. Wegen des Bedarfs an größeren Proteinmengen wurde die Kultivierung von *C. albicans* Hefe- und Hyphezellen in einem Fermenter etabiliert. Außerdem wurden auch sekretierte, intrazellulär prozessierte Peptide im Kulturüberstand von *C. albicans* Hyphenkulturen untersucht. Interessanterweise wurde ein Peptid, das Ece1p-III, identifiziert, welches als erstes zelllytische Pilzpeptidtoxin beschrieben wurde. Es zerstört die Epithelmembran des Wirtes und aktiviert die Epithelimmunität. Zusätzlich wurde Peptide des uncharakterisierten Proteins CaO19.3004 in größeren Menge im Überstand

nachgewiesen. Dessen Proteinsequenz enthält eine Fasciclin-Domaine und repetitive KR-Elemente ähnlich wie beim Ece1p Protein.

Im Weiteren fokussierte sich die Arbeit auf die serologischen Antworten des Wirts auf extrazelluläre Proteine von *C. albicans*. In systemisch, mit *C. albicans* infizierten Mäusen waren die serologischen Antworten auf sezernierte Proteine von *C. albicans* Hefezellen weniger ausgeprägt und vielfältig im Vergleich zur Antwort bei Menschen.

Des Weiteren wurde eine eingehende Untersuchung der serologischen Reaktionen von Candidämie-Patienten auf extrazelluläre Proteine von *C. albicans* durchgeführt. Zu diesem Zweck wurde ein 2D-Immunoblot Ansatz mit anschließender massenspektrometrischer Identifizierung von Proteinen verwendet. Basierend auf dem Screening von Seren von Candidämie-Patienten und drei Kontrollgruppen (Systemisches inflammatorisches Response-Syndrom, Bakteriämie und gesunde Probanden) wurden 20 immundominante, extrazelluläre Proteine identifiziert, wobei aus dieser Gruppe sieben Proteine potentielle diagnostische Marker für den Nachweis einer invasiven Candidämie darstellen (Xog1p, Rot2p, Eno1p, Met6p, Tsa1p, Tpi1p und Prx1p). Interessanterweise zeigten einige sekretierte, stark glykosylierte Proteinantigene hohe Kreuzreaktivitäten mit Seren aus Kontrollgruppen ohne *Candida* Infektion. Die Antikörpererkennung von Proteinen, die aus *C. albicans* Hyphezellen stammte, wurde nach enzymatischer Deglykosylierung erhebllich vermindert. Weiterhin bestätigte die enzymatische Deglykosylierung der rekombinant hergestellten, sekretierten Aspartylprotease Sap6p, dass Glycan-Epitope einen signifikanten Beitrag zur Erkennung von Sap6p durch Antikörper aus Patientenseren leisten.

Zusammenfassend liefert diese Studie neue Erkenntnisse über die serologische Reaktion von Candidämie-Patienten auf extrazelluläre Proteine des Hefepilzes *C. albicans* und seiner beiden morphologischen Wachstumsformen, Hefe- und Hyphezellen. Sieben extrazelluläre Antigene von *C. albicans* wurden als vielversprechende Biomarker Kandidaten zur Diagnose und Prognose von Candidämien identifiziert. Wie in dieser Arbeit gezeigt wurde, verstärkt die Glykosylierung extrazellulärer Proteine der Hefe *C. albicans* die Immunogenität und begünstigt zudem die unspezifische Bindung von Anti-Glykan Antikörpern, was zu Kreuzreaktivität mit Proteinantigenen von *C. albicans* führen kann.

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Introduction

1 The human pathogenic fungus *Candida albicans*

Candida albicans is a common fungal pathogen of humans, which can cause systemic, life-threatening infections. It is capable of growing as unicellular budding yeast cells, filamentous hyphae or pseudohyphae, which is termed polymorphism. There is no known soil life cycle for *C. albicans* as described for other fungi with a saprophytic lifestyle, such *as Aspergillus fumigatus, Histoplasma capsulatum* and *Paracoccidioides brasiliensis* (Klein *et al.* 2007; Gow *et al.* 2012). Instead, the natural ecological niche of *C. albicans* is the mammalian host, where it is primarily associated with the gastrointestinal tract.

Among the human pathogenic fungi, *C. albicans* is the most common cause of nosocomial bloodstream infections (Perlroth *et al.* 2007). In up to 70% of healthy individuals, *C. albicans* colonizes the mucosal surfaces as a commensal, such as genital and gastrointestinal mucosa (Gow *et al.* 2002; Casadevall *et al.* 2003; Saville *et al.* 2003; Wächtler *et al.* 2011; Gow *et al.* 2012). However individuals, lacking a proper immune response, are predisposed to superficial candidiasis of the oral or vaginal mycosal surface and under certain circumstances even to life-threatening disseminated candidemia, especially among immunocompromised patients. It has been reported that 50 to 75% of women in their childbearing years suffer from at least one episode of vulvovaginitis, and 5 to 8% have at least four episodes annually (Sobel 2007; Brown *et al.* 2012). In the US, the frequency of disseminated candidiasis was approximately 24 cases per 100,000 individuals in a year (Wilson *et al.* 2002; Perlroth *et al.* 2007). A very high mortality rate of up to 40% is often associated with systemic candidiasis (Alonso-Valle *et al.* 2003; Gudlaugsson *et al.* 2003; Pappas *et al.* 2003; Perlroth *et al.* 2007).

An intriguing trait of *C. albicans* is that this fungus is able to adapt to a diversity of host niches as a benign member of the microbial flora. However it can also infect host niches by the means of a wide range of virulence factors and fitness attributes. They include the morphological transition between yeast and hyphal form, the expression of adhesins and invasins, thigmotropism, the secretion of hydrolytic enzymes and the formation of biofilms (Mayer *et al.* 2013). During infection, yeast cells initiate hypha formation on epithelial cell surfaces (Figure 1). They penetrate

and invade the epithelium, once their filaments grow through the epithelial tissue and penetrate into blood vessels, where they switch back to yeast cells, disseminate with the bloodstream, adhere to endothelial cells again and penetrate into the organs nearby (Figure 1C).

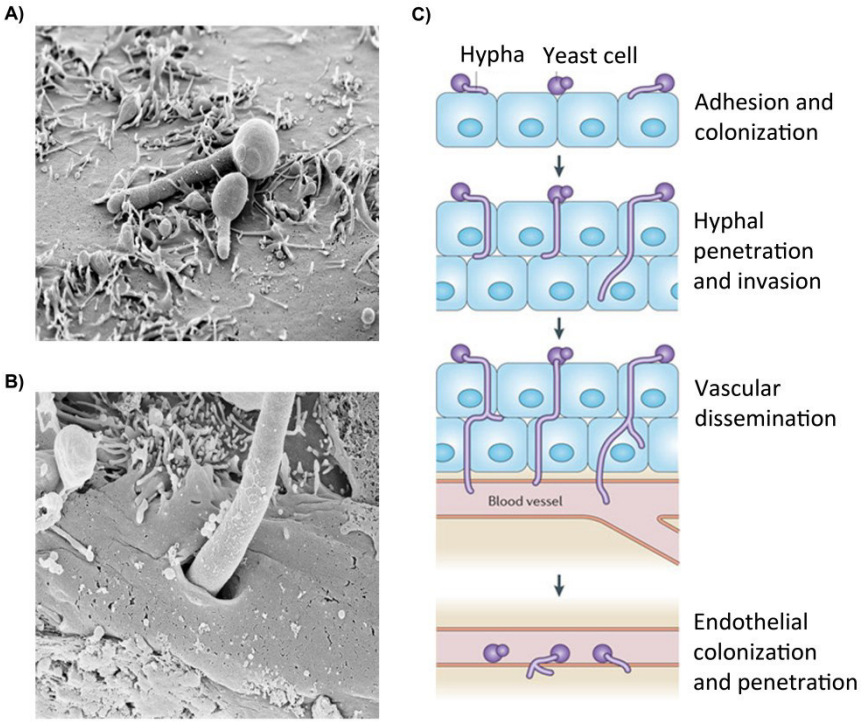


Figure 1 A) Electron microscopic image of *C. albicans* cells colonizing the oral mucosa. B) Electron microscopic image of a *C. albicans* hyphal filament penetrating through the oral mucosa. Photographs A and B courtesy: Holland/Özel, RKI. C) *C. albicans* tissue invasion. This picture is modified from *Nat. Rev. Microbiol.* 2012, Gow *et al.*

Since *C. albicans* lives as a commensal in the host, it is difficult to distinguish the infectious from the harmless commensal form during the diagnosis of a *Candida* infection. Still, there is lack of efficient and reliable diagnostic biomarkers for *C. albicans*. Until now, the blood culture is still the gold standard for the identification of systemic *Candida* infection in the clinics. In fact, the frequency of disseminated candidiasis is markedly underestimated with a proven positive blood culture as a single diagnostic criterion. Past autopsy studies and more recent clinical investigations showed that 30-50% of patients with disseminated candidiasis had negative blood cultures (Berenguer *et al.* 1993; Kami *et al.* 2002; Perlroth *et al.* 2007). To improve the early diagnosis of *Candida* infections, newer non-culture-based assays have been investigated as a tool for the detection of diagnostic

biomarkers, e.g. the antigens mannan, L/D-arabinitol, ß-glucan (Ostrosky-Zeichner 2012). According to the sensitivity and specificity the superior biomarkers are ß-1,3-D-glucan and mannan antigens (Held *et al.* 2013). However, they are still far from being efficient for a reliable diagnosis. Many factors can lead to false-positive results, such as contamination with fungal components in the albumin preparations or hemodialysis with cellulose membranes, which mimic the structure of ß-glucans (Ostrosky-Zeichner 2012). Therefore novel diagnostic *Candida*-specific antigens are still strongly needed.

For the efficient treatment of disseminated candidiasis, an early initiation of an effective antifungal therapy is crucial (Perlroth *et al.* 2007). In non-neutropenic patients fluconazole is a standard antifungal compound for disseminated candidiasis. However fluconazole is not efficient to treat *C. glabrata* infections, so antifungals with a broader spectrum of activity such as echinocandins (Caspofungin, Anidulafungin) or the triazole voriconazole are preferred. Nevertheless, the mortality of disseminated candidiasis is still high, and new therapeutic and preventative strategies are badly needed (Perlroth *et al.* 2007). In human host, extracellular proteins of *C. albicans* directly exposed to immune cells and also interacted with them.

2. Extracellular proteins of *C. albicans*

2.1 Classical secretory pathway in fungi

In fungi, like *C. albicans*, most secreted proteins enter the lumen of the rough endoplasmic reticulum as the first organelle of the secretory pathway *via* an N-terminal hydrophobic signal peptide. There, the secretory proteins are properly folded and post-translationally modified. Misfolded or incorrectly modified proteins are delivered to proteasomes (Hampton 2002) or vacuoles (Coughlan *et al.* 2004) for degradation. After the quality control of extracellular proteins, those who are properly folded are translocated to the Golgi complex, where they are further modified and packed in vesicles for the transport to the cell surface or secretion to the outer space. Vacuolar proteins, which are also sorted in the Golgi apparatus, are delivered to vacuoles *via* endosomes. In filamentous-growing fungi, the Golgi apparatus is often found at the apical regions of hyphae (Cole *et al.* 2000; Kuratsu *et al.* 2007), which supports the hypothesis that protein secretion mainly occurs at hyphal apices.

Transmembrane proteins and glycosylphosphatidylinositol (GPI)-anchored proteins are retained in the cell membrane or moved further to the cell wall and are covalently attached to glucans *via* a truncated GPI anchor (GPI-proteins) (Klis *et al.* 2002; Sorgo *et al.* 2013). The soluble secreted proteins are either continuously released (constitutive secretory pathway) or stored in granules for rapid discharge after certain stimulation (regulated secretory pathway) (Halban *et al.* 1994).

2.2 Nonconventional secretory pathways in fungi

During the past decade using the two yeast model organisms *S. cerevisiae* and *C. albicans,* more and more evidences of nonconventional secretory pathways have been provided by morphological, biochemical and genetic studies. They are comprehensively reviewed in Nombela *et al.* (2006). It could be clearly shown that many proteins lacking an N-terminal signal peptide reach the cell surface and extracellular milieu. These proteins are mainly involved in the cell-wall structure and dynamics, and interactions with host components.

Several non-conventional secretory pathways have been described in *S. cerevisiae* and *C. albicans*, which were comprehensively reviewed by LaJean Chaffin (Nombela *et al.* 2006; Chaffin 2008). In short, *ScSTE*6 encodes an ATP-binding cassette transporter that specifically exports the peptide pheromone α-factor (Magee *et al.* 2002). The nonclassical export genes *NCE101* and *NCE*102 are able to export the mammalian nonclassical substrate galectin-1, when it is expressed in yeast (Cleves *et al.* 1996). Endosome recycling is also proposed for the return of *S. cerevisiae* Ste3p back to the plasma membrane. This protein represents a classically exported a-factor pheromone receptor (Chen *et al.* 2000). Another postulated pathway of unconventional protein secretion is membrane blebbing, which involves the formation of exosomes on the outer surface of the cell, and membrane flip-flop, which describes a direct translocation across the plasma membrane (Nickel 2003). Other routes include substrate-specific interactions of helper proteins with the secreted proteins (FGF-1, IL-1α) (Prudovsky *et al.* 2003; Rajalingam *et al.* 2007; Rodrigues *et al.* 2008). Recently, extracellular vesicles with cytosolic proteins have been isolated from *C. albicans* and *Cryptococcus neoformans* (Rodrigues *et al.* 2008; Gil-Bona *et al.* 2015). Whatever mechanism underlies the nonconventional secretion,

they all directly or indirectly play a role in the interaction between the fungus and its host.

2.3 Proteolytic processing in the secretory pathway

In the late secretory pathway of eukaryotic cells, proteolytic processing is a major form of post translational modification, reviewed in Zhou *et al.* (1999). Many proteins are synthesized as inactive precursors. They may be called proproteins or zymogens in case of enzymes. After proteolytic processing these proproteins are activated. The process is often carried out by serine proteases, which remove a peptide segment from either end of the target protein or cleave internal bonds of the protein that lead to major changes in the structure and function of the protein. In yeast this process is very crucial for the activation, inhibition or destruction of numerous enzymes and peptide hormones (Bader *et al.* 2008). In mammals, many cellular processes are regulated by this process, like gene expression (Sakai *et al.* 1998), embryogenesis (Dubois *et al.* 1995; Cui *et al.* 1998; Peschon *et al.* 1998; Roebroek *et al.* 1998), the cell cycle (Zhou *et al.* 1999), programmed cell death (Thornberry *et al.* 1998) and endocrine/neural functions (Zhou *et al.* 1999).

The proteolysis is mainly mediated by calcium-dependent serine endoproteases. They are related to yeast subtilisin proteases (Steiner *et al.* 1992; Zhou *et al.* 1999). Therefore they are also called Subtilisin-like Proprotein Convertases (SPCs) or PCs. In mammals, the SPCs family consists of seven members (Seidah *et al.* 2006), while fungi harbor only a single gene, which encodes for a subtilisin-like serine proteinase and is designated as *KEX*2 (Bader *et al.* 2008). The phenotypes of *KEX*2 deletion mutants have been reported from many fungi, such as S*. cerevisiae* (Wickner 1974)*, P. pastoris* (Werten *et al.* 2005)*, C. albicans* (Newport *et al.* 1997; Newport *et al.* 2003)*, C. glabrata* (Bader *et al.* 2001)*, Schizosaccharomyces pombe* (Ladds *et al.* 2000)*, Aspergillus nidulans* (Punt *et al.* 2003). These characteristics include morphological changes and reduced virulence in *C. albicans* (Newport *et al.* 2003), hypersensitivity to antimycotic drugs in *C. glabrata* and inviability in *S. pombe* (Ladds *et al.* 2000; Bader *et al.* 2001). In general, fungal Kex2p proteases recognize specific lysine-arginine or arginine-arginine motifs in their substrate proteins. However, the structural features and the amino acids surrounding

the processing site contribute to the recognition as well (Bader *et al.* 2008). Besides a few well-known substrates of fungal Kex2p, such as α-pheromone precursors, killer toxin precursors and aspartic protease propeptides, some new substrates were found in *C. albicans:* The protein CaEce1, the uncharacterized CA0365, one member of the Pry protein family and a group of CaOps4-like proteins (Bader *et al.* 2008).

2.4 The cell wall proteins of *C. albicans*

The cell wall proteins of *C. albicans* are often released into the medium during growth (Sorgo *et al.* 2010). The cell wall of *C. albicans* is characterized by two distinguished layers. The out layer is composed of glycoproteins associated with mannan, while the inner layer consists of cross-linked polysaccharides, mainly including chitin, ß-1,6-glucan and ß-1,3-glucan (Figure 2).

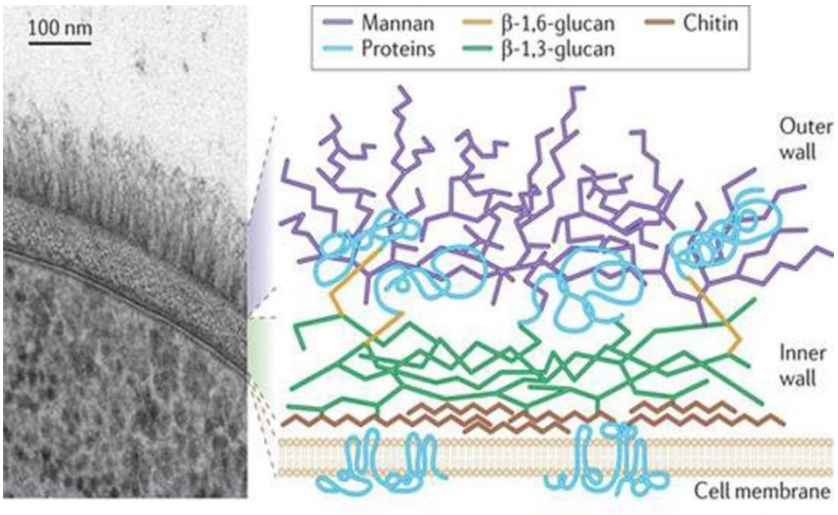


Figure 2 Structure of the *C. albicans* cell wall. Picture was modified from Gow *et al.,* 2012, *Nat. Rev. Microbiol*.

Cell wall proteins can be categorized into covalently attached proteins and non-covalently attached proteins. GPI-anchored proteins and Pir proteins (proteins with internal repeats) are the most abundant covalently attached protein classes. GPI-CWPs are linked to ß-1,6-glucan through a glycophosphatidylinositol remnant, whereas Pir proteins are directly linked to cell wall ß-1,3-glucan *via* an alkali-labile linkage (Figure 2). Proteins lacking a covalent attachment to the polysaccharide matrix may be heterogeneously distributed on the surface or secreted into the external milieu. These proteins may either be involved in cell wall organization, e.g., Bgl2p, have substrates within the cell wall, or released to the external milieu where they help taking up nutrients or mediating immunological interactions with host cells.

2.5 Glycosylation of extracellular proteins

Cell wall and secreted proteins of *C. albicans* are highly modified by the addition of carbohydrate structures, which consist of α- and ß-linked mannose units (Hall *et al.* 2013). The mechanism of mannosylation in *C. albicans* has been extensively summarized elsewhere (Hall *et al.* 2013). In brief, mannose monosaccharides polymerize into mannan and form distinguished structures: linear O-linked mannan, highly branched N-linked mannan and phospholipomannan. Protein mannosylation is initiated during protein synthesis in the endoplasmic reticulum (ER) and continues in the Golgi apparatus. Initially, mannose and glucose residues are added to acceptor proteins from dolichol phosphate (DP)-linked sugars, which are synthesized by dolichol phosphate mannose synthase (DPMS) and dolichol phosphate glucose synthase (DPGS), respectively. The core structure of N-mannan is a dolichol pyrophosphate-anchored oligosaccharide comprised of three glucose, nine mannose and two N-acetylglucosamine residues ($Glc_3Man_9GlcNAc_2$). It is transferred to nascent polypeptides in the ER by the oligosaccharyl transfer complex (OST) and processed by three glucosidases (Cwh41p, Rot2p and Mns1p), resulting in formation of the mature core ($Man_8GlcNAc_2$). The N-mannan core is structurally similar in all eukaryotes, but the outer N-mannan chain is fungal specific. In *C. albicans* mannosyltransferase Och1p catalyzed the attachment of mannan to the N-mannan core through an α-1,6 backbone to form branched outer chain mannan.

The *C. albicans* cell wall consists of 80-90% carbohydrates, which are predominantly associated with proteins on the outer layer. It is well known, that the cell wall carbohydrate mannan, ß-glucan and chitin are the major PAMPs presented to innate immune cells during infection. For example, TLR2, TLR4, dectin-2, Mincle, DC-SIGN and galectin-3 have major roles in the recognition of fungal mannans (Fradin *et al.* 2000; Tada *et al.* 2002; Porcaro *et al.* 2003; Taylor *et al.* 2004; Rouabhia *et al.* 2005; McGreal *et al.* 2006). Dectin-1 and complement receptor 3 are the major PRRs to detect ß-glucans (Thornton *et al.* 1996; Brown *et al.* 2001). Studies on a series of glycosylation mutants with altered mannan surface structures have shown that recognition of *C. albicans* is predominantly driven by mannan through TLR4 and mannose receptor (MR) in monocytes, while macrophage recognition is predominantly mediated by ß-glucan through dectin-1.

In addition, mannan and ß-glucan are also dominant antigens detected in systemic candidiasis patients. For this reason they are used as diagnostic biomarkers for *Candida* infections (Ostrosky-Zeichner 2012; Held *et al.* 2013).

3 The adaptive immune response

During host-fungal pathogen interaction, the innate immunity as the first defense line eliminates the majority of fungal pathogens within a short period of time. However, the adaptive immune response acts more specific and generates memory immunity for a long-term protection.

3.1 Structure of antibodies

One major function of the adaptive immunity upon fungal infection is generation of antibodies against fungal antigens. Antibodies are soluble proteins circulating freely and contributing to the immunity against foreign invaders, such as neutralization of toxins and microorganisms, recruitment of other components of the immune system for cellular cytotoxicity, immunomodulation and the generation of protective antifungal memory immunity (Janeway *et al.* 2005).

Antibody molecules are roughly Y-shaped molecules. They are generated by activated mature B cells in the secondary lymphoid organs (SLOs), such as the spleen and lymph nodes. In mammals, antibodies are divided into five isotypes, IgA, IgD, IgE, IgG and IgM. They basically consist of two large identical heavy chains and two small identical light chains. They are linked to each other by disulfide bonds. Both heavy and light chains are composed of constant and variable regions. The amino-terminals of both variable domains provide two identical specific antigen binding sites, whereas the constant domains can interact with effector molecules and cells. Each B cell only produces antibodies containing a unique variable chain. The variable region of each antibody heavy chain is encoded by the gene segments variable (V), diversity (D) and joining (J). The light chain contains only V and J segments. Antibodies have for each gene segment multiple copies. Each variable region is assembled by randomly selecting and combining one V, one D and one J gene segment. Thus different combinations of gene segments can lead to a huge

number of antibodies with different antigen specificities (Figure 3) (Janeway *et al.* 2005).

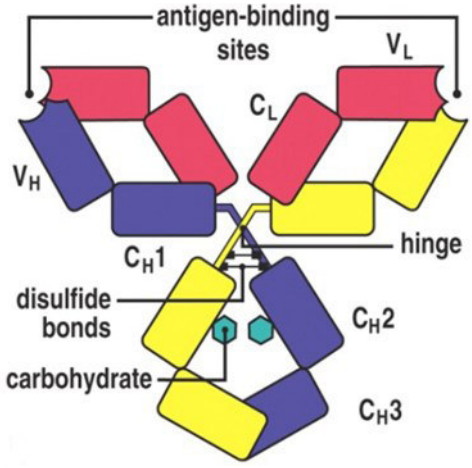


Figure 3 Schematic representation of the structure of an antibody molecule (adapted from Immunobiology, 6/e, Janeway, by Garland Science publishing)

3.2 Antibody generation

Host antibody response is common during host-fungal pathogen interaction. It occurs not only during active infections caused by pathogenic fungi, but also in response to normal exposure to environmental fungi and as a result of commensalism.

Upon infection, specialized antigen-presenting cells are activated, once they engulf a pathogen, which gets degraded intracellularly. Dendritic cells and B-cells (and to a lesser extent macrophages) are termed professional antigen-presenting cells (APC), which are capable of presenting antigens *via* major histocompatibility complex (MHC) molecules. Exogenous antigens are usually presented by APCs *via* MHC class II molecules, which activate $CD4^+$ T helper cells. While endogenous antigens are typically displayed on the MHC class I molecules, which are expressed by all host cells except non-nucleated cells, and activate $CD8^+$ cytotoxic T-cells (Janeway *et al.* 2005).

Naive T-cells are activated when their surface T-cell receptor (TCR) interacts with a peptide-bound MHC molecule. The activated T-cells differentiate into mature T-cells and secrete cytokines to attract more macrophages, neutrophils and other lymphocytes to infected cells. Like T cells, each B cell expresses a unique B cell receptor (BCR), which recognizes and binds to only one particular antigen. Once a B cell encounters its specific antigen in the lymph node, it engulfs the antigen and digests it. Then it displays antigen fragments bound to its unique MHC molecules.

This antigen can either be free-floating like secreted proteins or presented by APCs, such as dendric cells and macrophages. Also the antigens could be proteins, glycoproteins, polysaccharides, whole virus particles and whole bacterial cells. When a mature matching helper T-cell recognizes this antigen and interacts with it, further differentiation of B cell into an effector cell, known as a plasma cell, is triggered. Plasma cells can multiply and secrete antibodies. These antibodies bind to antigens, making them easier targets for phagocytes and trigger the complement cascade (Figure 4). About 10% of plasma cells develop into long-lived antigen-specific memory B cells, which could be called upon to respond quickly once the same pathogen re-infects the host (Janeway *et al.* 2005).

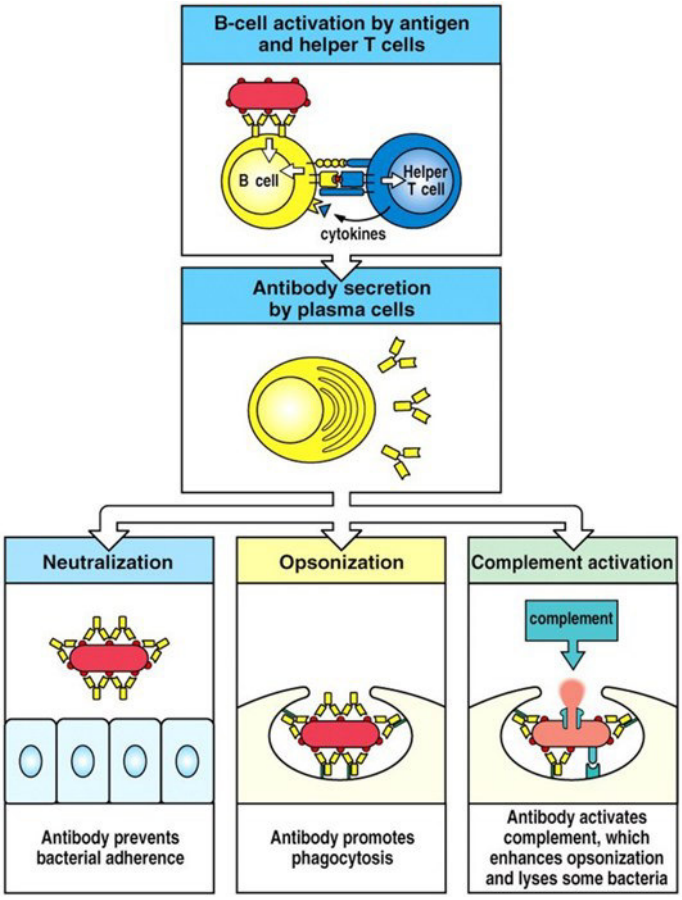


Figure 4 The humoral immune response is mediated by antibody molecules that are secreted by plasma cells (adapted from Immunobiology, 6/e, Janeway, by Garland Science publishing)

3.3 The serological response in candidemia patients and systemically infected mice with *C. albicans*

The serological response in candidemia patients reflects the formed antibodies in patient serum in response to a systemic *C. albicans* infection. The serological survey of molecular imprints of pathogens is typically applied to the diagnosis of an infection. In the last decades, technologies in the fields of proteomics and antibody engineering have progressed rapidly. 2D-gel based immunoproteomic approaches and microarray techniques largely facilitate identification of *Candida* antigenic proteins in serum samples of candidemia patients and infected mice (Pitarch *et al.* 2004; Pitarch *et al.* 2006; Pitarch *et al.* 2009; Pitarch *et al.* 2009; Mochon *et al.* 2010; Pitarch *et al.* 2011; Pitarch *et al.* 2014).

As *C. albicans* is a commensal on the human gastrointestinal mucosal surface, there is a basal level of natural antibodies circulating in healthy individuals. Until now, the identified immunodominant protein antigens of *C. albicans* are mainly cell wall components and glycolytic enzymes, including Eno1p, mannans and Mp65p. A 5-IgG antibody-reactivity signature (Anti-Met6p IgG, anti-Ssb1p IgG, anti-Gap1p/Tdh3p IgG, anti-Hsp90p IgG and anti-Pgk1p IgG antibodies) was suggested as a prognostic predictor for invasive candidiasis (Pitarch *et al.* 2011). The ß-1,3-glucosyltransferase Bgl2p was demonstrated to represent a putative vaccine candidate (Gil-Bona *et al.* 2015). Besides protein antigens, cell wall carbohydrates are also found to be highly immunoreative, such as mannan. Anti-mannan antibodies are prevalent in human sera, including patients, but also the general population (Lopez-Ribot *et al.* 2004). Anti-mannan antibodies can mediate a protective response in mice with invasive candidiasis (Han *et al.* 2000). Combined detection of the antigen mannan and anti-mannan antibodies has shown diagnostic value for *Candida* infection (Sendid *et al.* 2002).

4 The aim of the work

In this work, we aimed at characterizing the extracellular proteins (peptides) present in the culture supernatant of *C. albicans* yeast and hyphal cells. In order to characterize the constituents and changes of the secretomes during yeast-to-hypha

transition, we analysed *C. albicans* yeast and hyphal secretomes by a 2D-GE as well as an LC-MS/MS- based proteomic approach. Besides, we also investigated the peptides present in the supernatant of *Candida* cultures. For this purpose, solid phase extraction was applied for the enrichment of peptides from culture supernants.

Further, extracellular proteins (peptides) of *C. albicans* are exposed to immune cells of the host and present a direct antigen pool for APCs in the human humoral immunity. Still little is known about the interplay of *C. albicans* secreted proteins and the host humoral immune response. Until now, there are no effective and specific molecular diagnostic biomarkers for the detection of systemic candidiasis. Thus, the present work aimed to investigate the serological response profile of candidemia patients to secreted proteins of *C. albicans*. A further aim was to find a core set of anti-*C. albicans* protein antibodies which could be used as biomarkers for the diagnosis of *C. albicans* infection. 2D-immunoblotting of extracellular proteins combined with mass spectrometry for protein identification was applied to achieve this goal.

Materials and methods

1 Strains and materials

1.1 Strains

The strains used in this work are listed in Table 1.

Table 1 Fungal strains

Strain name	Genotype	Reference
Candida albicans		
SC5314	Wild type, clinical isolate	(Gillum *et al.* 1984)
Pichia pastoris		
pCA2	GS115 + pKJ113 with *C. albicans SAP*2 integration	(Borg-von Zepelin *et al.* 1998) (Schild *et al.* 2011)
pCA6	GS115 + pKJ113 with *C. albicans SAP*6 integration	

1.2 Media and buffers

The composition of media and buffers used in this study is described in Table 2. For growth of microorganisms on solid media, 2% (w/v) agar was added.

Table 2 Media and Buffers

Medium	Components
***Candida albicans* media**	
YPD rich medium	1% (w/v) yeast extract 1% (w/v) Bacto peptone (BD Biosciences) 2% (w/v) D-glucose
YNBS medium pH4.0	0.17% (w/v) yeast nitrogen base (YNB, without amino acids and ammonium sulfate, BD Biosciences) 5 g/L ammonium sulfate 2% (w/v) sucrose 100 mM sodium citrate buffer, pH 4.0
YNBS medium pH7.4	0.17% (w/v) yeast nitrogen base (YNB, without amino acids and ammonium sulfate, BD Biosciences) 5 g/L ammonium sulfate 2% (w/v) sucrose 5 mM GluNAc 75 mM MOPS, pH 7.4
***Pichia pastoris* media**	
BMG medium	100 mM potassium phosphate buffer, pH 6.0 1.34% (w/v) YNB with ammonium sulfate (BD Biosciences) 4 $\times 10^{-5}$ % (w/v) biotin

	1% (v/v) glycerol
	As BMG, but 0.5% (v/v) methanol instead of 1% glycerol
BMM medium	LB medium 1% (w/v) Bacto tryptone (BD Biosciences)
	0.5% (w/v) yeast extract
	1% (w/v) NaCl
***Escherichia coli* media**	
Buffers	
PBS pH 7.4	140 mM NaCl
	2.7 mM KCl
	10 mM Na_2HPO_4
	1.8 mM KH_2PO_4
Sodium citrate buffer 0.1 M pH 4.0	0.1 M citric acid : 0.1 M sodium citrate (59:41)
MOPS buffer 75 mM pH 7.4	Adjust pH to 7.4 with NaOH

1.3 Serum samples

1.3.1 Mouse serum samples

In this work mouse serum samples were used for the investigation of the serological response of mice against secreted proteins of *C. albicans* yeast cells. The mouse serum samples used in this work are described in table 3. Here the classical systemic infection model was applied in almost all the mice experiments except for Ca3-10. In brief, *C. albicans* wild type, mutant or complemented strains were intravenously injected into the tail vein of immunocompetent female BALB/c mice. For mice survival experiments, an infectious dose of 1×10^4 /g was applied and mice were sacrificed if moribund, whereas a higher infectious dose of 2.5×10^4 /g was used for fungal burden experiment and mice were sacrificed at predefined time points. In microevolution experiments two mice per passage were challenged intravenously with 5×10^3 *C. albicans* cells per gram body weight. After 14 days of infection *C. albicans* cells were removed from the infected kidney and passed to the next passage of mice. The details of above described mice experiments are described in Lüttich *et al.* (Luttich *et al.* 2013) and Hennicke *et al.* (Hennicke *et al.* 2013). Besides, in the Ca3-10 mice experiment, the intraperitoneal model was used. Briefly, 1×10^8 *C. albicans* cells / 500 µl were injected intraperitoneally, mice were sacrificed after 12 and 24 h. More details about this infection model are described in the reference (Thewes *et al.* 2007). In all animal experiments, the German animal protection law was followed and experiments were approved by the responsible Federal State

authority (Thüringer Landesamt für Lebensmittelsicherheit und Verbraucherschutz) and ethics committee.

Table 3 Mouse serum samples

Mouse experiments	**Genotype** (*C.albicans* SC5314)	**Cage/animal**	**Lifetime** (day)
Ca1-10 (10/02-24/02/2010) Microevolution, systemic infection, Infectious dose: 5×10^3 /g	wt	S7	14
	wt	S8	14
	wt	S11	14
Ca1-11 (27/01-17/02/2011) Fungal burden, systemic infection, Infectious dose: 2.5×10^4 /g	wt	2/8	2
	*ssu1*Δ	3/4	5
	SSU compl.	6/7	5
Ca3-11 (01/04-21/04/2011) Survival experiment, systemic infection, Infectious dose: 1×10^4 /g	wt, CAI4+pClp10	1/5	7
	*efg*Δ*cph*Δ (Evo 0)	3/2	20
	*efg*Δ*cph*Δ (Evo 0)	3/4	20
	*efg*Δ*cph*Δ (Evo 0)	4/7	20
	Evo X	5/1	8
	Evo X	6/8	7
	Evo X	6/9	14
Ca4-11 (03/04-07/04/2011) Fungal burden, systemic infection, 2.5×10^4 /g	wt, CAI4+pClp10	1/1	2
	wt, CAI4+pClp10	2/6	4
Ca5-11(29/03-19/04/2011) Survival experiment, systemic infection, Infectious dose: 1×10^4 /g	wt	1/3	8
	wt	1/5	8
	*ssu*1Δ	4/6	9
	SSU compl.	6/6	7
	*cdg*Δ	7/5	20
	*cdg*Δ	8/8	11
	CDG compl.	9/5	14
	CDG compl.	10/8	12
Ca3-10 (12/07/2010) intraperitoneal model	wt	B	20 min
PBS control			

1.3.2 Human serum samples

Serum samples, derived from six patients with severe sepsis due to candidemia and five patients with sterile systemic inflammatory response syndrome (SIRS) due to acute pancreatitis were used. These samples originated from the large collection of biomaterial of patients with sepsis of the Hellenic Sepsis Study Group (Registry reference www.sepsis.gr). Enrolled patients were hospitalized at the 4th Department of Internal Medicine and at the 2nd Department of Critical Care Medicine of ATTIKON University Hospital, at the Intensive Care Units of Tzaneion Piraeus Hospital and of Laikon Athens General Hospital and at the 2nd Department of Internal Medicine of Sismanogleion Athens General Hospital. The registered study protocols

were approved by the Ethics Committee of the hospitals and written informed consents were provided by the first degree relatives because patients were not able to consent. Blood was sampled within the first 24 hours from onset of signs of SIRS. Patients with neutropenia, primary immunodeficiency, HIV infection and chronic intake of corticosteroids were excluded from the study. Six individual serum samples without clinical or microbiological evidence and with a similar age to candidemia cases were collected from the University Hospital Jena after institutional approval by Local Ethics Committee Jena and evaluated as negative controls. Written informed consents were given by all the patients. Base-line characteristics of the study patients and controls are shown in Table 4.

Table 4 Base-line characteristics of the study patients and controls

Characteristic	Candidemia	Negative controls		
	C.albicans **(n=5)**	**SIRS patients (n=5)**	**Bacteremia (n=5)**	**pre-OP[3] patients (n=6)**
Demographic factors				
Sex				
Male	3	1	2	3
Female	2	2	3	4
Age (median)	64	58	71	64
≤65 years	3	2	1	4
>65 years	2	1	4	3
Positive blood culture (*C. albicans*)	5	/	/	/
Bacterial Infection	3	/	5	/
Underlying diseases				
Solid tumor	1	/	n.a.[4]	n.a.
Respiratory dysfunction	3	/	n.a.	n.a.
Gastrointestinal pathology	/	4	n.a.	n.a.
Gallbladder stones	1	/	n.a.	n.a.
Acute pancreatitis	/	4	n.a.	n.a.
Heart failure	1	/	n.a.	n.a.
COPD[1]	3	/	n.a.	n.a.
Nephrolithiasis	/	1	n.a.	n.a.
Risk factors for SC[2]				
Latrogenic predisposing factors				
Broad spectrum antibiotics	5	1	n.a.	n.a.
Immunosuppressive therapy	1	/	n.a.	n.a.
Central venous catheters	3	/	n.a.	n.a.
Parenteral nutrition	3	/	n.a.	n.a.
Underlying malignancy	1	/	n.a.	n.a.
Outcome of hospital stay				
Death	1	/	1	n.a.
Discharge	4	5	4	n.a.

1: COPD: Chronic obstructive pulmonary disease;
2: SC: systemic candidiasis; 3: Pre-OP: before operation; 4: n.a.: not applicable

1.3.3 Recombinant *C. albicans* Sap6p expressed in *Pichia pastoris*

Recombinant *C. albicans* Sap6p was expressed in *P. pastoris* (*Komagataella pastoris*) (Invitrogen) and purified by cation exchange chromatography on an SP-sepharose column, followed by desalting through a Sephadex G25 column. The protein sample was resolved in 0.1 M sodium citrate buffer, pH 4.5 as described in previous studies (Borg-von Zepelin *et al.* 1998; Schild *et al.* 2011).

2 Organism and growth conditions

2.1 *C. albicans* growth conditions

C. albicans strain SC5314 was used throughout this study (Gillum *et al.* 1984). Yeast cells were pre-cultured overnight in YPD medium on a rotary shaker with 200 rpm and at 30 °C and then transferred to flasks containing YNBS medium (pH 4). Pre-cultures in YNBS (OD=1) were used to inoculate a fermenter (Sartorius Stedim biotech Biostat® D-DCU 10L, Göttingen, Germany) for a batch cultivation of *C. albicans* yeast or hyphal cells.

2.2 Fermenter cultivation of *C. albicans*

For batch cultivation of yeast cells, a fermenter containing YNBS (5 g/L ammonium sulfate, 1.7 g/L nitrogen base, 20 g/L sucrose and 75 mM citric acid – sodium citrate buffer, pH 4) was inoculated with *C. albicans* yeast cells at an initial OD_{600} of 0.05. Cultivation was performed in a culture volume of 6 L at 30 °C, at a constant pressure of 400 mbar and at a maximum stirring rate of 400 rpm for 15 hours. For hyphal induction, 6L YNBS medium [5 g/L ammonium sulfate, 1.7 g/L nitrogen base, 20 g/L sucrose, 75 mM MOPS [3-(N-morpholino) propanesulfonic acid] (pH 7.4), supplemented with 5 mM N-acetylglucosamine (GlcNAc) was inoculated with a preculture of *C. albicans* yeast cells as described above at a temperature of 37 °C, a constant stirring rate of 100 rpm and a continuous pressure of 400 mbar for 18 hours.

2.3 Viability assay of *C. albicans* cells

Once *C. albicans* cells were harvested, the morphology of the cells was checked under the light microscopy to ensure the presence of one single morphotype, yeast or hyphae. Also the viability of the cells was monitored by fluorescent microscopy by means of propidium iodide staining. Propidium iodide with 1 mg/ml was added to 3 ml culture to give a final concentration of 10 µg/ ml. After 15 minutes of incubation in the darkness, the samples were checked by fluorescence microscopy. The detection of signals was performed according to the standard protocol of red fluorescent protein (RFP).

3 Protein biochemical methods

3.1 Isolation of secreted proteins from growth media

For the isolation of extracellular proteins, *C. albicans* cells were spun down at 12,000 × *g* and the supernatant was filtered with a 0.2 µm filter (PES, Millipore). Secreted proteins were precipitated overnight at 4 °C by the addition of 10 % (w/v) trichloroacetic acid (TCA) and 3 mg/ml DTT. After centrifugation for 1 hour at 30,000 × *g* at 4 °C the supernatant was discarded and protein pellets were washed two times with 90% (v/v) ice-cold acetone and centrifuged at 13,000 × *g* for 1 hour at 4°C. The pellets were air-dried for 10 min and resuspended in 2D lysis buffer [7 M urea, 2 M thiourea, 4% (w/v) CHAPS, 0.8% (v/v) pharmalyte (GE healthcare Life Sciences)] for 2D gel electrophoresis as described in Wartenberg *et al.* (2011). After solubilization of the protein pellet, samples were centrifuged at 20,000 × *g* for 15 min at room temperature and the supernatant was transferred to a new microcentrifuge tube. The protein concentration was measured using the Bio-Rad Protein Assay-Dye Reagent Concentrate (Bio-Rad, Munich).

3.2 Gel-based Proteomics

3.2.1 Two-dimensional gel electrophoresis (2D-GE)

For yeast and hyphal secretome analyses, 2D gel electrophoresis was carried out as described in Wartenberg *et al.* (2011) with slight modifications. The amount of 350 µg proteins per sample was loaded *via* sample cups on 24 cm IPG strips

(Immobiline DryStrip, GE Healthcare, Germany) that covered a pH range of either 4-7 or 7-11NL (non-linear). Isoelectric focusing was performed using an IPGphor II device (GE Healthcare, Germany) with the following program: 4 h for gradient 300V, 4 h for gradient 600V, 4 h for gradient 1000V, 4 h for gradient 8000V and 48,000Vhs at 8000V. Then, IPG strips were equilibrated with SDS equilibration buffer containing 1% (w/v) DTT for 15 min with gentle agitation, followed by equilibration with SDS equilibration buffer containing 2.5% (w/v) iodoacetamide. The second dimension was performed using self-casted 12.5% (w/v) polyacrylamide Tris/HCl gels and an Ettan Dalt Six electrophoresis unit (GE Healthcare, Germany). Gel electrophoresis was performed at 15 W/gel at 20 °C for 4 hours. Gels were stained with colloidal Coomassie G250 (Dyballa *et al.* 2009).

3.2.2 Protein identification using MALDI-TOF/TOF analysis

Excised protein spots were in-gel digested with trypsin as described in Shevchenko *et al.* (1996). The resulting peptides were extracted from gel pieces with extraction buffer (50% ACN in 0.05% TFA) and resuspended in an ultrasonic bath.

For MALDI-TOF/TOF analysis samples were reconstructed in 10 mg/ml HCCA solved in solution [30% / 70% (v/v) ACN / 0.1% TFA]. The samples were measured on an ultrafleXtreme MALDI-TOF/TOF device (Bruker Daltonics, Germany). MS spectra (m/z 700-3500 Da) were acquired and the 10 most intense precursor ions were selected for MS/MS fragmentation. FlexControl 3.3 software was used for data collection and flexAnalysis 3.3 for spectra analysis and peak list generation. The generated MS and MS/MS spectra were analyzed by searching the *Candida* Genome Database (http://www.candidagenome.org/) using MASCOT 2.3 server (Matrix Science, UK). Search parameters were the following: oxidized methionine as variable modification, carbamidomethyl cysteine as fixed modification, one miscleavage and a pepide mass tolerance of 100 ppm and a fragment mass tolerance of 0.6 Da. Results were regarded as significant with an allowed likelihood for a random hit of $p \leq 0.05$, according to the Mascot score. Three to four biological replicates of secretome samples from each morphological form were analyzed.

3.2.3 Protein identification using LC-MS/MS analysis

Samples not identified by MALDI-TOF-TOF, were further analyzed by LC-MS/MS. They were solved in 10 µL aqueous 1% (v/v) formic acid. Depending on the

protein amount, 5-10 µL of the sample were injected into LC-MS/MS system consisting of a nanoAcquity UPLC system (Waters, Manchester, UK) on-line coupled to a Synapt HDMS tandem mass spectrometer (Waters, Manchester, UK). LC-MS/MS data were collected using data-dependent acquisition (DDA). Each acquisition cycle consisted of a survey scan covering the range of m/z 400-1500 amu followed by MS/MS fragmentation of the four most intense precursor ions collected over a 1 sec interval in the range of 50-1700 m/z. Dynamic exclusion was applied to minimize multiple fragmentations for the same precursor ions.

DDA raw files were collected using MassLynx v4.1 software and processed using ProteinLynx Global Server Browser (PLGS) v.2.5 software (Waters, Manchester, UK) under baseline subtraction, smoothing, deisotoping, and lockmass-correction. Pkl files of MS/MS spectra were generated and searched against NCBInr database (updated December, 05, 2012, installed on a local server) using MASCOT version 2.4. Mass tolerances for precursor and fragment ions were 15 ppm and 0.03 Da, respectively. Other search parameters were: instrument profile, ESI-Trap; fixed modification, carbamidomethyl (cysteine); variable modification, oxidation (methionine); up to one missed cleavage was allowed.

3.3 Gel-free LC-MS/MS analysis

3.3.1 Protein sample preparation

The secretome of *C. albicans* was also characterized by LC-MS/MS analysis. Protein pellets obtained after TCA precipitation were resolubilized in 50 µl 50 mM NH_4HCO_3 in 50%/50% (v/v) trifluoroethanol /water and ultrasonicated for 15 min and subsequently denaturated at 90 °C for 15 min. Cysteine residues were reduced by addition of 5 µL 200 mM DTT and incubation for 20 min at 50 °C. Subsequently, reduced cysteine residues were carbamidomethylated for 30 min at room temperature in the dark by applying 5 µL 1 M iodoacetamide. Afterwards, samples were diluted approximately 1:10 with 445 µl 50 mM NH_4HCO_3 (pH 8) to gain a final TFE concentration of <5 %. Proteins were digested for 18 h at 37 °C with Trypsin-LysC mix (Promega, USA) at a ratio of 1:25 trypsin to protein. The reaction was stopped by acidification with formic acid. Finally, samples were dried in a SpeedVac

and resolubilized in 20 µL 0.05% TFA in 98%/2% (v/v) water/ACN for LC-MS/MS measurement.

3.3.2 LC-MS/MS analysis

LC-MS/MS analysis was carried out on an Ultimate 3000 nano RSLC system coupled to a QExactive Plus mass spectrometer (both Thermo Fisher Scientific). Peptides were enriched and desalted online based on a pre-concentration set-up using a nano-trap column (Acclaim Pep Map 100, 2 cm x 75 µm, 3 µm) at a flow rate of 5 µL/ min. After 4 min, a valve switch was performed to elute the pre-concentrated peptides onto the analytical column. For separation of peptides, an Acclaim Pep Map RSLC column (15 cm × 75 µm, 2 µm) was used as stationary phase (Thermo Fisher Scientific). The binary mobile phase consisting of A) 0.1% (v/v) formic acid in H_2O and B) 0.1% (v/v) formic acid in 90/10 ACN/H_2O was applied for 135 min gradient elution: 0-5 min at 4% B, 85 min at 30% B, 95 min at 45% B, 100-109 min at 90% B, 110-135 min at 4% B. LC performance was routinely checked by cytochrome C standard digest and blind injections prior and after analysis and by monitoring UV absorption at λ = 214 nm during analysis. The Nano-spray Flex Ion Source (Thermo Fisher Scientific) provided with a stainless steel emitter was used to generate positively charged ions at 2.0 kV spray voltage. The capillary temperature was set to 220 °C and the S-lens RV level to 50. The mass spectrometer was calibrated externally prior to the analysis with positive CalMix solution and internally based on the lock mass of m/z 445.12003 amu for $[C_2H_6SiO]_6$ (polydimethylsiloxane). The hybrid quadrupole/orbitrap mass analyzer was operated in Full MS / dd MS2 (TopN) mode. Precursor ions were measured in full scan mode within a mass range of m/z 350-1700 at a resolution of 35k FWHM using a maximum injection time of 120 ms and an AGC (automatic gain control) target of 1e6. For data-dependent acquisition, up to 10 most abundant precursor ions per scan cycle with an assigned charge state of z = 2-4 were selected in the quadrupole for further fragmentation using an isolation width of m/z 2.0. Fragment ions were generated in the HCD cell at a normalized collision-energy of 30 V using nitrogen gas. Dynamic exclusion of precursor ions was set to 30 s. The LC-MS/MS instrument was operated by means of the Thermo/Dionex Chromeleon Xpress v6.80 SR13 build 3818 software and the Thermo QExactive Plus Tune /Xcalibur v3.0.63 2.3 build 1765 graphical interface software.

3.3.3 Bioinformatic analysis

The CGD (www.candidagenome.org) database was used for database searches. Search algorithms of Mascot v2.4.1 (Matrix Science, UK), Sequest HT and MS Amanda were used to identify proteins. Two missed cleavages were allowed for trypsin digestion. Oxidation of Met and carbamidomethylation of Cys were considered as dynamic and static modifications, respectively. Percolator node and a reverse decoy database was used for (q-value) validation of the peptide spectral matches (PSMs) using a maximum Delta CN of 0.05 and a strict target false discovery (FDR) rate of < 1%. At least two peptides per protein identified in two replicates were required for positive protein hits. The LC-MS/MS proteomics data were deposited to the ProteomeXchange Consortium (Vizcaino *et al.* 2014) *via* the PRIDE partner repository with the dataset identifier PXD003201 and 10.6019/PXD003201. Identified proteins were classified by using the GO Slim Term (http://www.candidagenome.org/cgi-bin/GO/goTermMapper) according to their biological processes. Signal peptide prediction was done by using SignalP4.1 Server (http://www.cbs.dtu.dk/services/SignalP/). To predict the GPI modification sites the program Big PI Predictor was applied (http://mendel.imp.ac.at/gpi/gpi_server.html).

3.4 Quantitative analysis of *C. albicans* secretome *via* TMT labeling

For quantitative analysis of *C. albicans* secretome, three biological replicates of yeast secretome samples and hyphal samples were respectively labeled with TMT6plex tags. For each experiment two technical replicates were done and for each replicate three measurements were performed.

3.4.1 Protein sample preparation

Protein pellets obtained after TCA precipitation and 3 washing steps with 90% acetone, were resolubilized in 150 µl denature buffer [45 mM TEAB (triethyl ammonium bicarbonate) + 0.1% SDS + cOmplete protease inhibitor cocktail/PhosSTOP 1 tablet for 10 ml] in 50%/50% (v/v) trifluoroethanol /water and ultrasonicated for 15 min and subsequently denaturated at 90 °C for 15 min. Cysteine residues were reduced by addition of 5 µL reduction buffer [200 Mm TCEP (tris-2-carboxyethylphosphine) in 200 mM TEAB] and incubation for 60 min at 55 °C. Subsequently, reduced cysteine residues were carbamidomethylated for 30 min at

room temperature in the dark by applying 5 µL alkylation buffer (375 mM Iodacetamide in 100 mM TEAB). Then proteins were precipitated from the solution using methanol/chloroform method. Protein pellets were dried and resolubilized in 100 µl 100 mM TEAB solution. Proteins were digested for 18 h at 37 °C with Trypsin-LysC mix (Promega, USA) at a ratio of 1:25 trypsin to protein. After that samples were ready and prepared for TMT labeling.

Each TMT6plex labeling vial (0.8 mg) was resolublized with 41µl ACN. Each protein sample was transferred to one TMT6plex-vial, as described in the supplement table 5. After 1 hour of incubation at room temperature the reactions were stopped by the addition of 5% hydroxylamine in 180 mM TEAB for 15 min incubation time. Subsequently, all the 6 labeled samples were mixed together in a new microcentrifuge tube. Finally, samples were dried in a SpeedVac and resolubilized in 20 µL 0.05% TFA in 98/2 (v/v) water/ACN for LC-MS/MS measurement

3.4.2 LC-MS/MS analysis and bioinformatic analysis

The samples were analyzed by LC-MS/MS as described in chapter 3.3.2 and 3.3.3.

3.5 2-DE and immunoblot analysis

For immunoblots, 80µg protein samples were loaded on 11 cm IPG strips (GE Healthcare) covering a pH range of 3-11 NL (non-linear). The program for isoelectric focusing was as following: 3h for gradient 300V, 4h for gradient 600V, 4h for gradient 1000V, 4h for gradient 8000V and 24,000Vhs at 8000V. After equilibration of IPG strips as described, the gel electrophoresis was performed by using any kD™ Criterion™ TGX Stain-Free™ gels (Bio-Rad, Germany). Gels were scanned by the Gel Doc EZ system (Bio-Rad, Germany). Proteins were transferred to the PVDF membrane with low fluorescent background (Immobilon® FL-Membrane, Merck Millipore, US) at 0.6 A for two hours by tank blotting (Trans-Blot® Cell, Bio-Rad). Blots were blocked with 1% (w/v) casein (Western blocking reagent, Roche, Switzerland) in TBST washing buffer [TBS containing 0.1% (v/v) Tween 20] for 1 hour, followed by incubation with human serum at a 1:1000 dilution for 1 hour. After three washing steps, blots were scanned by the Bio-Rad Gel Doc EZ system. The upper left corner of the blotting membrane was cut off and both parts of the blot were separately

incubated for 1 hour with an anti-IgG secondary antibody (Goat anti-Human Immunoglobulin G antibody, HRP conjugate, Merck Millipore, US) at a dilution of 1:100,000 and 1:20,000, respectively. After three washing steps, immunoblots were covered with the Immuno-Blue fluorescent substrate (NH DyeAGNOSTICS, Germany). Fluorescent signals were visualized by using a VersaDoc MP 4000 imaging system (Bio-Rad, Germany). Gel images, protein imprint on the membrane blot and signal images of immunoblots were compared by means of the gel imaging software Delta 2D 4.5 (Decodon, Germany).

3.6 Glycoprotein staining

For detection of glycoproteins in PA gels the Glycoprotein staining kit (G-Biosciences, USA) was used.

3.7 Deglycosylation of recombinant Sap6p protein and secretome samples

The Protein Deglycosylation Mix (New England BioLabs, Ipswich, MA) was applied for deglycosylation of recombinant proteins and secretome samples.

3.8 Peptides enrichment *via* SPE

For SPE experiment, the preculture of *C. albicans* was carried out as described in chapter 2.1. The induction of hypha formation was performed in shaking flasks for 18 hours at 37 °C and shaking at 200 rpm.

The medium supernatant was sterile filtered through a PES membrane with a pore size of 0.2 µm. To the filtered supernatant, an EDTA-free cOmplete protease inhibitor tablet (1 tablet per 100ml, Roche), EDTA, pH 8 (0.4 mM) and benzamidine (1 mM) were added. Peptides were enriched based on a two-step solid phase extraction (SPE) enrichment protocol using first a C4 resin. The flow-through was subsequently enriched on a C18 resin. For every enrichment process, each column was firstly wetted with 5 ml acetonitrile (ACN), followed by 5 ml 0.05% trifluoroacetic acid (TFA) equilibration, sample loading, washing with 5 ml 2% ACN and 0.05%TFA and elution with 80% ACN and 0.05% TFA. The eluate after C4 and C18 enrichment

steps was collected in microcentrifuge tubes and dried in speed-vac. Before LC-MS/MS measurement the sample was filtered with 10kDa MWCO spin filters, dried in a vacuum centrifuge and resolubilized in loading solution [0.2% formic acid in 71:27:2 ACN/H_2O/DMSO (v/v/v)].

3.9 ELISA assay for the determination of sera IgG titers directed against *C. albicans* secreted proteins

0.1 µg *C. albicans* hyphal proteins were coated on the bottom of wells of a 96-microtiter plate with carbonate-bicarbonate buffer, overnight at 4 °C with mild shaking. The wells were washed 3 times with TBST [0.1% (v/v) Tween 20] buffer and subsequently blocked with 1% (w/v) casein in TBS buffer for 2 hours. Then, washing steps were repeated. Patients´ sera were diluted 1:1000 in blocking solution and incubated for 30 min at room temperature. The patients´ sera that were used in this ELISA assay are listed and described in the supplementary table S-4. After repeating the washing step goat anti-human IgG secondary antibodies, which were diluted 1:50,000 in blocking solution, were added and incubated for 30 min at room temperature. After a final washing step, 50 µl /well TMB were added as chromogenic substrate and incubated for 15 minutes. The color reaction was stopped by the addition of 2 M H_2SO_4 (50µl/well). Subsequently the microtiter plate was analyzed by using a spectrophotometer at a wavelength of 450nm.

Results

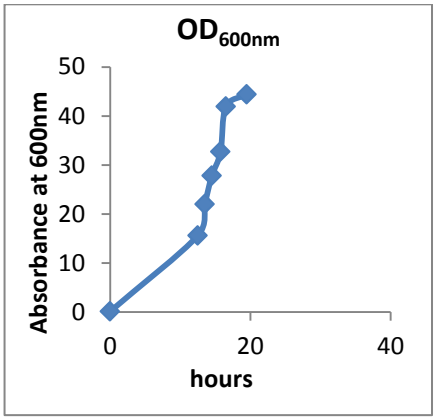
1 Cultivation of *C. albicans* in a fermenter

C. albicans yeast cells usually do not secrete a large number of proteins into the medium (Sorgo *et al.* 2010). To obtain the required amount of protein for 2D gel-based secretome analysis we established a batch cultivation of *C. albicans* yeast and hyphal cells, respectively, to overcome the limitations of shaking flask cultures. *C. albicans* yeast cells were cultivated in the YNB medium (pH4) and hypha formation were induced by the addition of GlcNAc as inducer and increasing the temperature and pH. During the whole cultivation, the pH and temperature were monitored and kept constant. The morphology and viability of *C. albicans* cells were checked under the microscope after cell harvest. Only one morphotype (yeast or hypha) was detectable in the corresponding cultures.

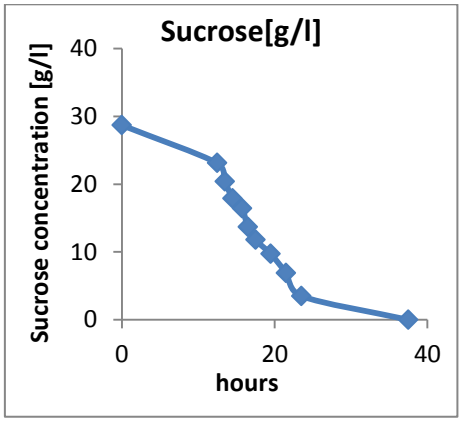
1.1 Growth curve of *C. albicans* yeast cells during fermentation

In order to investigate the growth behavior of *C. albicans* yeast cells in a 10 L fermenter, a preliminary fermentation experiment was carried out for 3 days. Figure 5A shows that *C. albicans* yeast cells reached the end of the exponential growth phase after 20 hours cultivation based on the development of the cell density. Figure 5B shows that the sucrose in the growth medium was completely depleted after 3-day cultivation. Figure 5C illustrates that nitrogen (NH_4^+) and phosphate (PO_4^{3-}) were still present in sufficient amounts for effective growth after 3 days of fermentation. The plotting of the CO_2 concentration released by *C. albicans* yeast cells reached its maximum after 15 hours of fermentation (in the supplement Figure S1). The CO_2 evolution rate usually correlates well with the yeast growth kinetics. For this reason, we set the fermentation time of *C. albicans* yeast cells to 15 hours, which allowed us to harvest viable, actively growing *C. albicans* cells.

A.



B.



C.

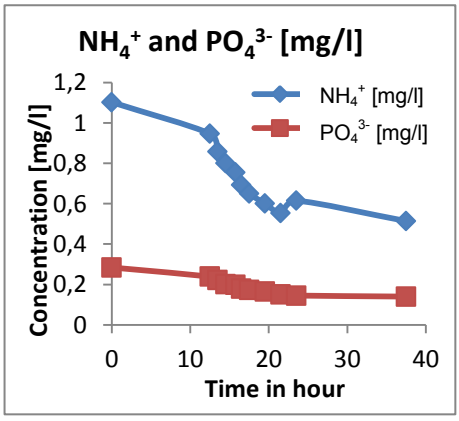


Figure 5. Growth curve of *C. albicans* yeast cells and consumption of sucrose, nitrogen and phosphate. A) Development of cell density within 20 hours of fermenter cultivation; B) Consumption of sucrose; C) Consumption of nitrogen (NH_4^+) and phosphate (PO_4^{3-}) in the medium during a three-day cultivation

1.2 Growth curve of *C. albicans* hyphal cells during the fermentation

We observed the dynamics of the biomass development of *C. albicans* hyphal cells during the cultivation for 4 days in a 10 L fermenter. Figure 6 shows that hyphal cells were growing in a linear mode during the 4-day cultivation period. Within the linear phase a short interval of reduced growth appeared. Due to quorum sensing, the induced hyphal cells of *C. albicans* may switch back to yeast cells when a certain cell density is reached. Indeed, the microscopic monitoring of *C. albicans* cells revealed that hyphal cells start to switch back to the yeast form after 18 hours of

fermentation. For this reason we set the fermentation time for *Candida* hyphal cells to 18 hours.

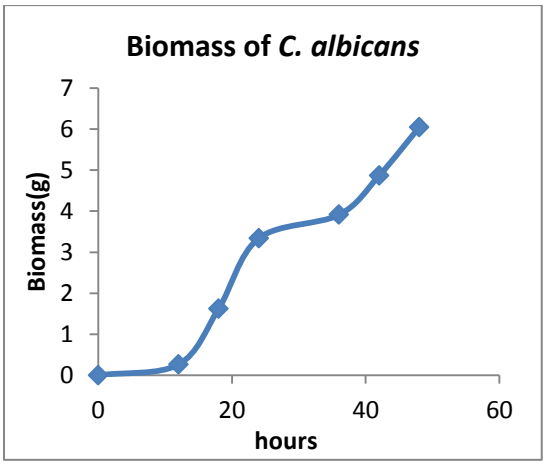


Figure 6 Growth curve of *C. albicans* hyphal cells during batch cultivation.

1.3 Viability test of *C. albicans* cells

After each cell harvest, we checked the morphology and viability of cells by light and fluorescent microscopy. Propidium iodide was applied for viability staining. Figure 7 shows that almost all yeast cells were vital, while hyphal cells clumped together and a small amount of hyphal cells (less than 10% of the total number of cells) showed a positive propidium iodide fluorescence signal.

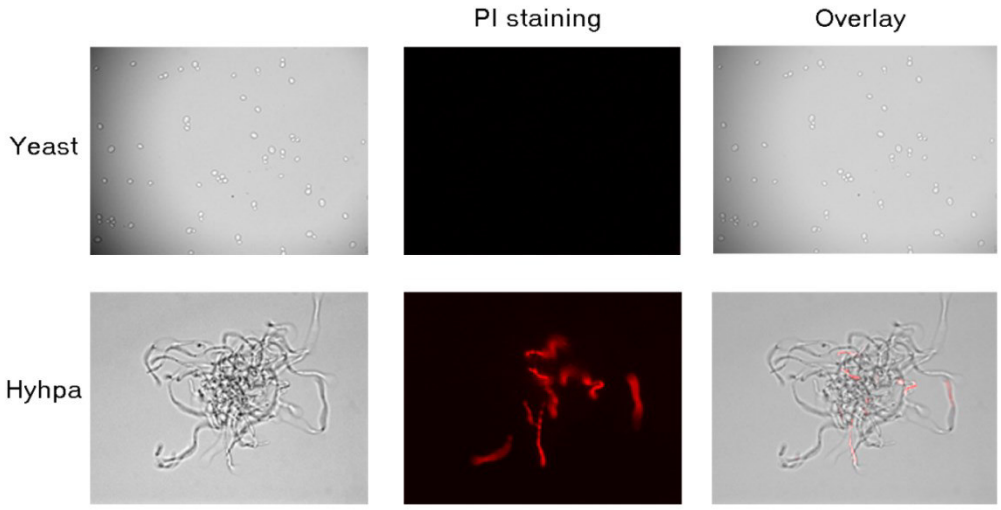


Figure 7 Cell viability assays of *C. albicans* yeast and hyphal cells after fermentation. Dead cells were detected by propidium iodide fluorescence staining.

2 Secretome analysis of *C. albicans* during a yeast-to-hypha transition

To obtain a comprehensive secretome map of *C. albicans*, both 2D gel based- and LC-MS/MS based-proteomic approaches were applied for the analysis the secretomes of *C. albicans* yeast and hyphal cells. The secreted protein samples were prepared by precipitation of proteins from the supernatant of *C. albicans* culture with TCA. After several washing and drying steps the protein pellets were resuspended in either urea sample buffer for 2D-GE or trifluoroethanol buffer for LC-MS/MS proteomic analysis. 2D gel-based and LC-MS/MS-based secretome analysis were both carried out with four biological replicates of yeast and hyphal secretome samples, respectively.

2.1 2D gel-based secretome analysis

For a better spatial separation of protein spots, IPG strips with a pH range of 4-7 and 7-11 were applied. Notably, no protein spots were detected in a pH range of 7 to 11 on 2D gels of secreted yeast proteins, while on 2D gels covering a pH range of 4-7, some high abundant protein spots could not be separated well, which occupied a rather large area in the upper-left corner. These spots turned out to mainly consist of the mannoprotein Mp65p and other abundant cell wall proteins like Sun41p, Tos1p and Kar2p (Figure 8A).

2D-gel electrophoretic separation of the hyphal secretome revealed that most secreted proteins have an acidic pI, but a small fraction of alkaline proteins was also detectable, mainly Sap5p and Sap6p (Figure 8B). Of a total of 579 excised protein spots, 412 were identified by MALDI-TOF/TOF representing 22 different yeast proteins and 141 different hyphal proteins. Spot labeling and protein identification are listed in the supplement Figure S-3 and Table S-1. The identified extracellular proteins are mainly associated with stress responses, carbohydrate metabolic processes and transport. 33 out of 155 identified proteins contained an N-terminal signal peptide for secretion based on *in silico* prediction and three proteins exhibited a predicted GPI anchor attachment signal.

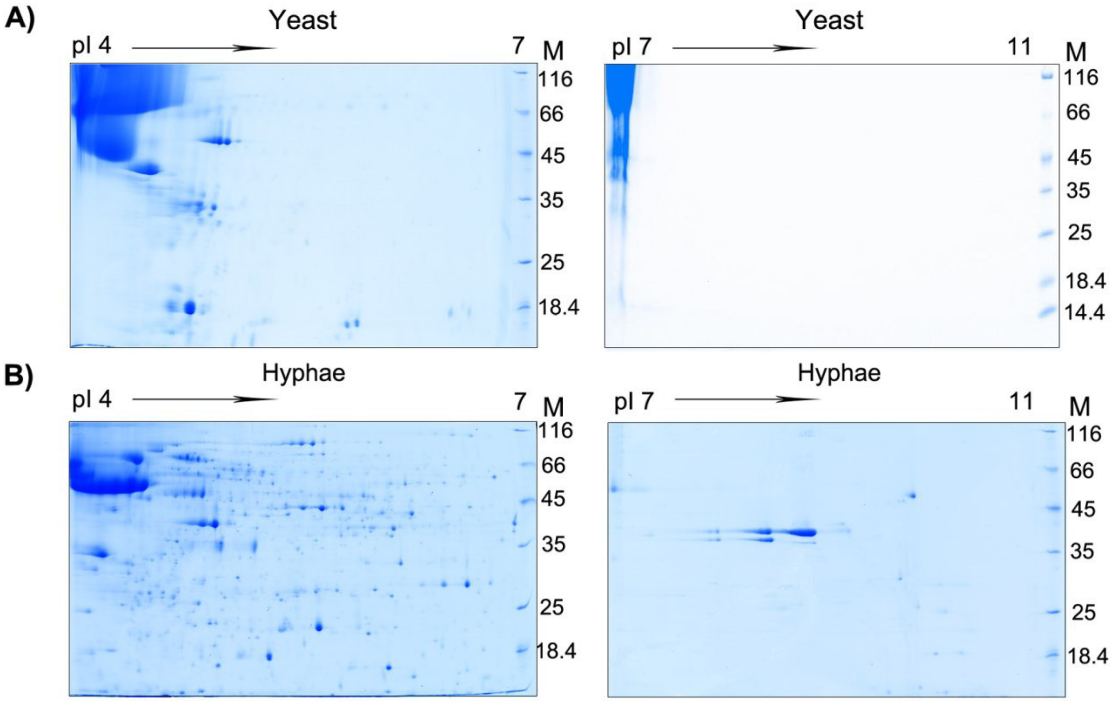


Figure 8 2D maps of the *C. albicans* yeast and hyphal secretome. A) 2D secretome map of *C. albicans* yeast cells; B) 2D secretome map of *C. albicans* hyphal cells. The pH gradient is indicated and the molecular weight of the protein marker is given.

2.2 Gel-free secretome analysis of *C. albicans*

2.2.1 Qualitative analysis of *C. albicans* secretome

For LC-MS/MS-based characterization of the *C. albicans* secretome, proteins from four biological replicates were analyzed by LC-MS/MS in three technical replicates. A total of 83 yeast proteins and 364 hyphal proteins were identified in at least three biological replicates with at least two peptides identified in more than two of three technical replicates (Supplement Table S-1). These proteins were mainly associated with regulation of biological processes, response to stress and chemicals, transport, translation and carbohydrate metabolic processes (Figure 9). Hyphal cells secreted more proteins than yeast cells, particularly proteins related to translation. Among them, 106 proteins contained an *in silico* predicted signal peptide and 21 a predicted GPI anchor (Supplement Table S-1).

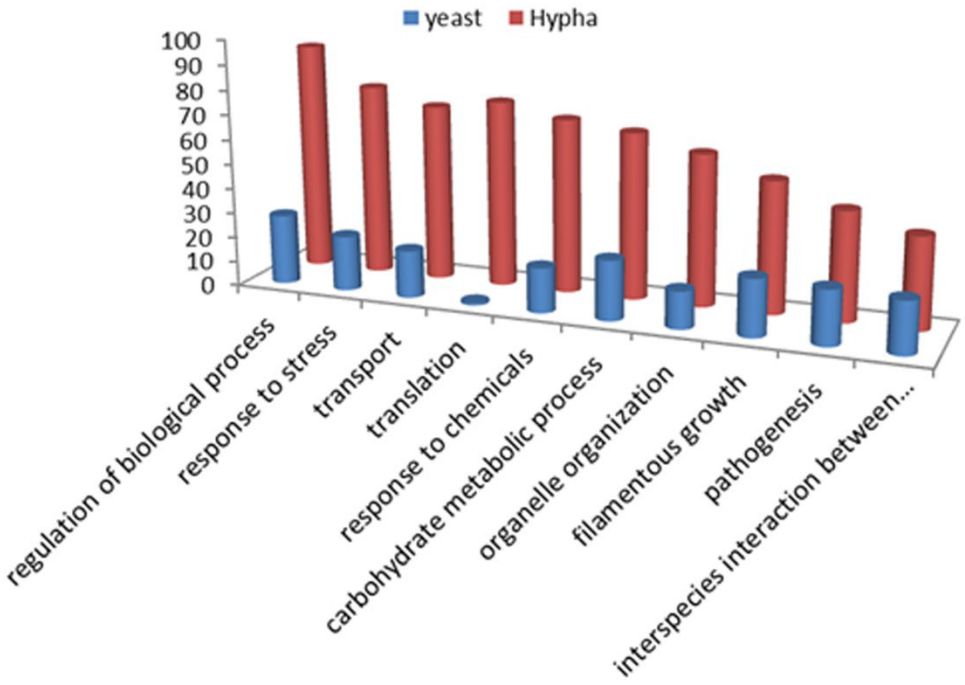


Figure 9 Categorization of secreted yeast and hyphal proteins identified by LC-MS/MS according to their potential biological process using CGD Gene Ontology slim mapper.

2.2.2 Quantitative analysis of *C. albicans* secretome

Tandem mass tag TMTsixplex labeling was applied for quantitative analysis of *C. albicans* secretome. The relative abundance of secreted proteins, which were shared by yeast and hyphal cells, were determined *via* TMT reporter ions. Therefore, the ratio of abundance of a certain protein allows to determine the relative changes (-fold changes) of this secreted protein during the yeast-to-hypha transition. The protein amount was normalized to the biomass, in our case to the cell dry weight. Only 7 proteins were more than 2-fold abundant in yeast than in hyphal cells, whereas 155 proteins were more than 2 fold enriched in hyphal in comparison to yeast cells (supplement Table S-2). Proteins Ape3p, Rbt1p, Als3p, Rct1p and Abg1p were the most 5 “upregulated” secreted proteins during the yeast-to-hypha transition, whereas Ywp1p, Dag7p, Als4p, Plb4.5p and Rbe1p were the top 5 most “downregulated” proteins in hyphae (Table 5)

Table 5 The top 5 of most "up- and downregulated" proteins during yeast-to-hypha transition

Average Ratio Hyphae/yeast	Ratio Hyphae/Yeast Normalized to biomass	Accession	Gene	Protein Description	Σ# PSMs*
↑ 2,230	↑ 5,945	orf19.3591	*APE3*	Putative vacuolar aminopeptidase Y	296
↑ 2,200	↑ 5,866	orf19.1327	*RBT1*	Cell wall protein with similarity to Hwp1	197
↑ 2,182	↑ 5,819	orf19.1816	*ALS3*	Cell wall adhesin	277
↑ 2,109	↑ 5,623	orf19.7350	*RCT1*	Fluconazole-induced protein	65
↑ 2,036	↑ 5,430	orf19.1597	*ABG1*	Vacuolar membrane protein	204
↓ 0,044	↓ 0,117	orf19.3618	*YWP1*	Secreted yeast wall protein	89
↓ 0,156	↓ 0,415	orf19.4688	*DAG7*	Secretory protein; a-specific, alpha-factor induced	114
↓ 0,171	↓ 0,455	orf19.4555	*ALS4*	GPI-anchored adhesin	28
↓ 0,172	↓ 0,459	orf19.1442	*PLB4.5*	Phospholipase B	414
↓ 0,178	↓ 0,474	orf19.7218	*RBE1*	Pry family cell wall protein	104

* Σ#PSMs: the total number of identified peptide spectra matched for the protein

2.3 Comparison of the composition of the *C. albicans* yeast and hyphal secretome

Combining the data of 2D gel- and LC-MS/MS-based proteomic methods led to the detection of a total of 101 extracellular yeast and 410 extracellular hyphal proteins (Figure 10A). 65 proteins were shared by both cell morphologies, while 36 proteins were exclusively detected in yeast cells and 345 in hyphal cells (Figure 10A). The secreted yeast and hyphal proteins were categorized according to their biological process using CGD Gene Ontology slim mapper. In the yeast secretome, 39 cell wall-associated proteins (38 %), 11 proteins in response to environmental stimuli (11 %), 9 secreted proteases, lipases and adhesins (9 %), 8 carbohydrate metabolism-associated proteins (8 %) and 6 vesicle-mediated transport proteins (6 %) represented the major fraction of all secreted proteins (Figure 10B). By contrast, the hyphal secretome was enriched in proteins of the categories: cell wall-associated (18 %), translation (16 %), response to stimuli (13 %), carbohydrate metabolic process (9 %), and regulation of biological function (7 %) (Figure 10D). Altogether, 60 extracellular yeast (59 % of all identified proteins) and 86 hyphal proteins (21 % of all identified proteins) were predicted as secretory proteins based on the presence of an N-terminal signal peptide for secretion (SignalP4.1 server prediction). Among these proteins, 14 and 16 contained GPI-anchor attachment signals in yeast and hyphal cells, respectively (Figure 10C).

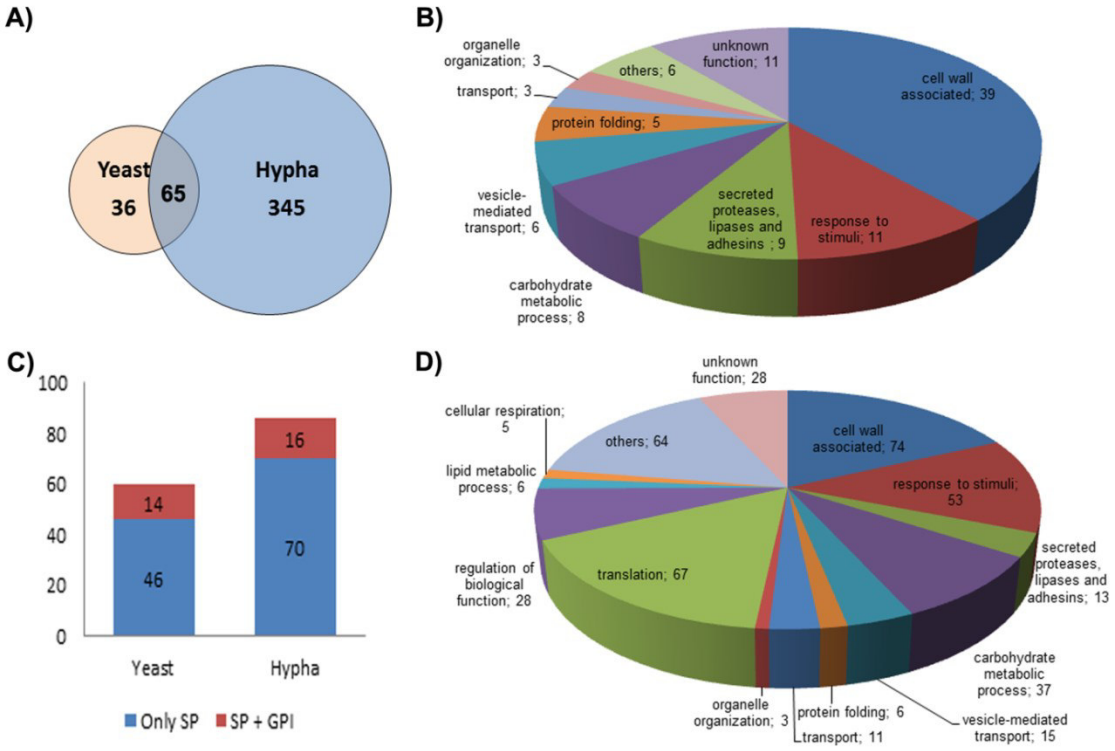


Figure 10 Comparison of the *C. albicans* yeast and hyphal secretome. A) Venn diagram for description of the numbers of identified secreted proteins in *C. albicans* yeast and hyphal cells; B) and D) Functional categorization (biological process) of the secreted protein in yeast (B) and hyphal cells (D); C) Overview of proportion of classical secretory proteins with a predicted SP (signal peptide) and GPI-anchor attachment in *C. albicans* secretomes. The secreted yeast and hyphal proteins were categorized according to their biological process using CGD Gene Ontology slim mapper.

2.4 Peptide enrichment of *Candida* hyphal secretome

Microbial secretomes consist not only of proteins, but also intracellularly processed peptides, which usually have important biological functions, such as anti-microbial, hormone-like or toxin-like effects (Bischofberger *et al.* 2012; Los *et al.* 2013). In order to investigate intracellular processed peptides and small proteins in the *C. albicans* secretome we applied solid phase extraction with C18 columns for peptide extraction from the culture supernatant of *C. albicans* hyphal cells. A total of 16 processed proteins were identified (Supplement Table S-3). Among them, Ece1p was the most abundant processed protein detected in the supernatant of hyphal cultures. It had almost a ten times higher abundance than the second most abundant peptide Sap9p. The top 5 of the most abundant processed proteins were Ece1p,

Sap9p, Rbt1p, orf19.3004 and Sap6p (Table 6). The amino acid sequence of Ece1p contains eight discontinuous KR amino residues. It is predicted that they are recognized and processed by a Kexin-like proteinase (Bader *et al.* 2008). Here, all the eight processed peptides were detected and identified by LC-MS/MS (Figure 11). Among these eight processed peptides, the Ece1p-III peptide was detected as the most abundant peptide (Supplement Table S-3).

Table 6 Protein list of identified processed proteins in the culture supernatant of *C. albicans* hyphal cells enriched by SPE

Accession	Description	ΣCoverage	Σ# Proteins	Σ# Unique Peptides	Σ# Peptides	Σ# PSMs*
orf19.3374	Ece1	88.56	1	54	54	256
orf19.6928	Sap9	5.88	1	3	3	23
orf19.1327	Rbt1	4.02	1	3	3	21
orf19.3004	orf19.3004	12.44	1	3	3	6
orf19.5542	Sap6	6.46	2	2	2	5
orf19.1597	Abg1	6.25	1	1	1	4
orf19.2452	orf19.2452	3.13	1	1	1	4
orf19.6741	orf19.6741	3.13	1	1	1	3
orf19.3642	Sun41	2.63	1	1	1	2
orf19.5585	Sap5	5.5	1	1	1	1
orf19.1442	Plb4.5	2.28	1	1	1	1
orf19.1426	orf19.1426	2.12	1	1	1	1
orf19.894	orf19.894	1.15	1	1	1	1
orf19.1490	Msb2	1.06	1	1	1	1
orf19.3681	orf19.3681	1.03	1	1	1	1
orf19.663	Gin4	0.89	1	1	1	1

* Σ#PSMs: the total number of identified peptide spectra matched for the protein

Signal peptide: MKFSKIACATVFALSSQA
Pep-I: AIIHHAPEFNMKR
Pep-II: DVAPAAPAAPADQAPTVPAPQEFNTAITKR
Pep-III: SIIGIIMGILGNIPQVIQIIMSIVKAFKGNKR
Pep-IV: EDIDSVVAGIIADMPFVVRAVDTAMTSVASTKR
Pep-V: DGANDDVANAVVRLPEIVARVATGVQQSIENAKR
Pep-VI: DGVPDVGLNLVANAPRLISNVFDGVSETVQQAKR
Pep-VII: DGLEDFLDELLQRLPQLITRSAESALKDSQPVKR
Pep-VIII: DAGSVALSNLIKKSIETVGIENAAQIVSERDISSLIEEYFGKA

Figure 11 Amino acid sequence of Ece1p processed by Kex2p

Intriguingly, the fourth most abundant processed protein, orf19.3004 also contains a similar KR repeat domain and an additional Fasciclin domain (FAS) (Figure 12). In the KR repeat domain, each predicted by Kex2p processed peptide contains the KNL conserved residues at almost the same position. The FAS domain, which is an ancient cell adhesion domain common in plants, animals and bacteria, is rich in the cell entry motif KXLK (Huber *et al.* 1994; Ulstrup *et al.* 1995) (Figure 12).

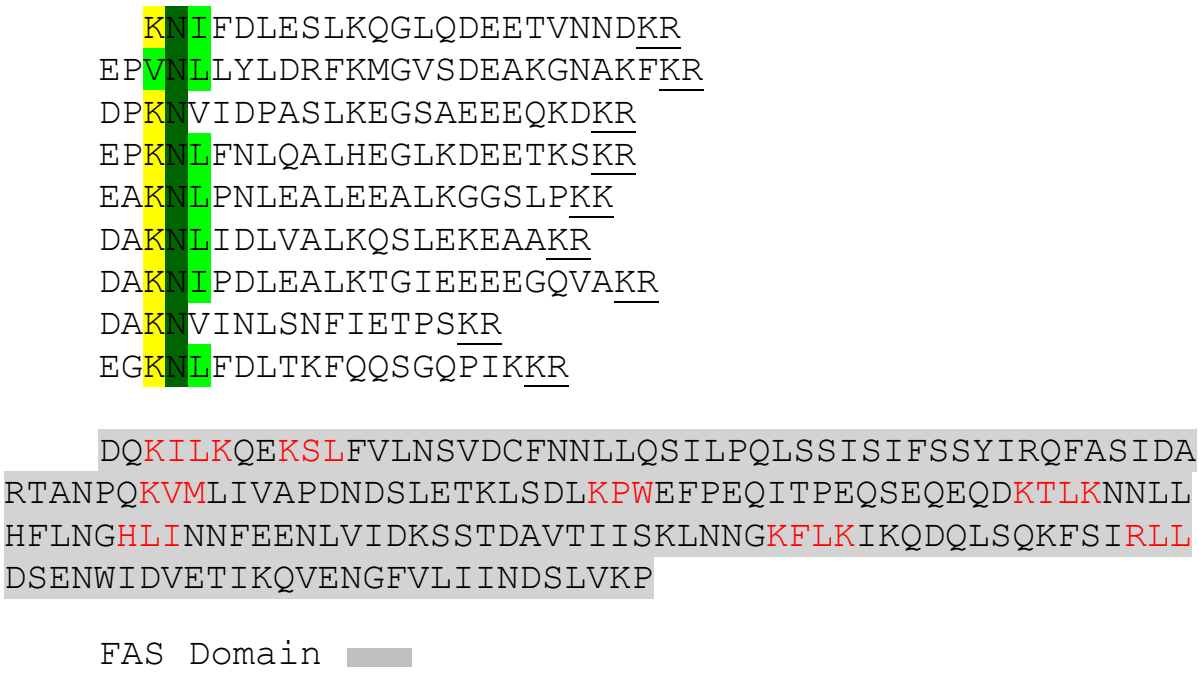


Figure 12 Amino acid sequence of orf19.3004 processed by Kex2p

3 Immunoproteomic study of *C. albicans* secretome during yeast-to-hypha transition

3.1 Serological response of mice to *C. albicans*

3.1.1 Generation of IgG antibodies against *C. albicans* in mice

In order to investigate the kinetics of *C. albicans*-specific IgG antibody generation in mice, we tested mice sera on different days after intravenous injection of *C. albicans* wildtype or mutants into the tail vein. Starting from the fifth day mouse sera contained *C. albicans*-specific antibodies in contrast to mice challenged with

PBS (Figure 13). This indicates that it needs at least 5 days to complete the production of specific IgG antibodies upon the infection with *C. albicans*.

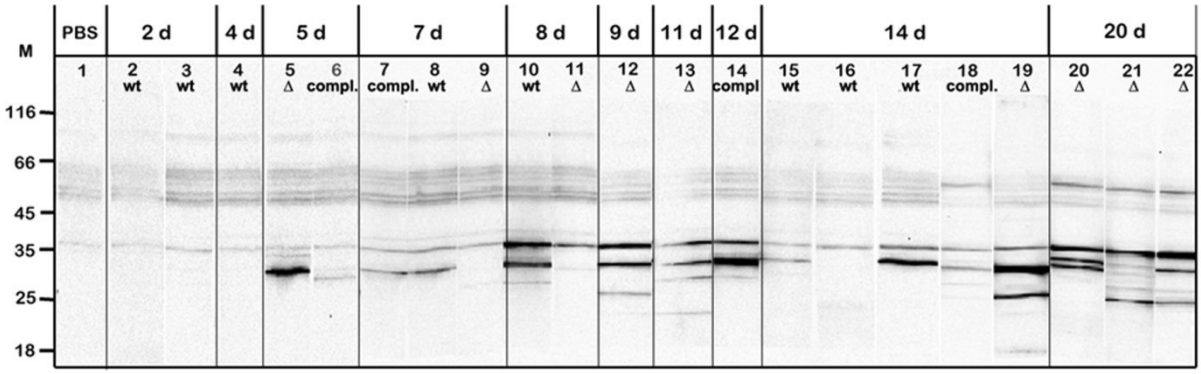


Figure 13 Generation of antibodies in time in mice upon infection with *C. albicans* wild type and virulence attenuated mutant strains. Here the number of lanes was marked and the used *C. albicans* strains were also indicated. wt: wild type; compl.: the correspondent complement strain, as described in Table 7. Δ**:** mutant strain, as described in the table 7.

Table 7 List of mouse sera, which were used in the corresponding lanes of the immunoblot shown in Figure 13. The details of mouse serum samples are described table 3.

Nr.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
Candida strain	**wt**	**wt**	**wt**	**wt**	***ssu1*Δ**	***SSU* compl.**	***SSU* compl.**	**wt**	***efg*Δ *cph*Δ**	**wt**	***efg*Δ *cph*Δ**
Mice sera	PBS	Ca1-11 2/8	Ca4-11 1/1	Ca4-11 2/6	Ca1-11 3/4	Ca1-11 6/7	Ca5-11 6/6	Ca3-11 1/5	Ca3-11 6/8	Ca5-11 1/3	Ca3-11 5/1
Nr.	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Candida strain	***ssu*1Δ**	***cdg*Δ**	***CDG* compl.**	**wt**	**wt**	**wt**	***CDG* compl.**	***efg*Δ *cph*Δ**	***cdg*Δ**	***efg*Δ *cph*Δ**	***efg*Δ *cph*Δ**
Mice sera	Ca5-11 4/6	Ca5-11 8/8	Ca5-11 10/8	Ca1-10 S7	Ca1-10 S8	Ca1-10 S11	Ca5-11 9/5	Ca3-11 6/9	Ca5-11 7/5	Ca3-11 3/2	Ca3-11 3/4

3.1.2 Serological response of mice to secreted proteins of *C. albicans* yeast cells

Figure 14A shows that the IgG antibody response of mice infected with a *C. albicans* wild type (SC5314) strain for 8 days is almost as low as observed in control sera, which were derived from mice infected for 20 minutes. The *C. albicans* double mutant Δ*efg1*/*efg* Δ*cph1*/*cph* that is unable to produce hyphae was used for infection experiments as well. It is characterized by an attenuated virulence (Samaranayake *et al.* 2013). Mice infected with this mutant strain survived and a

strong serological response directed against *C. albicans* yeast secreted proteins after 20 days of infection was elicited (Figure 14C). However, it differed from the responses observed in candidemia patients. Since *C. albicans* is a common commensal in humans, but not in mice, strong IgG antibody signals were detectable against the *C. albicans* yeast secretome even in healthy humans (Figure 14B). In most of candidemia patients, the serological anti-*C. albicans* IgG antibody profiles are more complex and variable in comparison to healthy individuals. In both healthy human individuals and candidemia patients, Mp65p and ß-glucanase Xog1p were identified as the dominant immunoreative antigens in this study (Figure 14B).

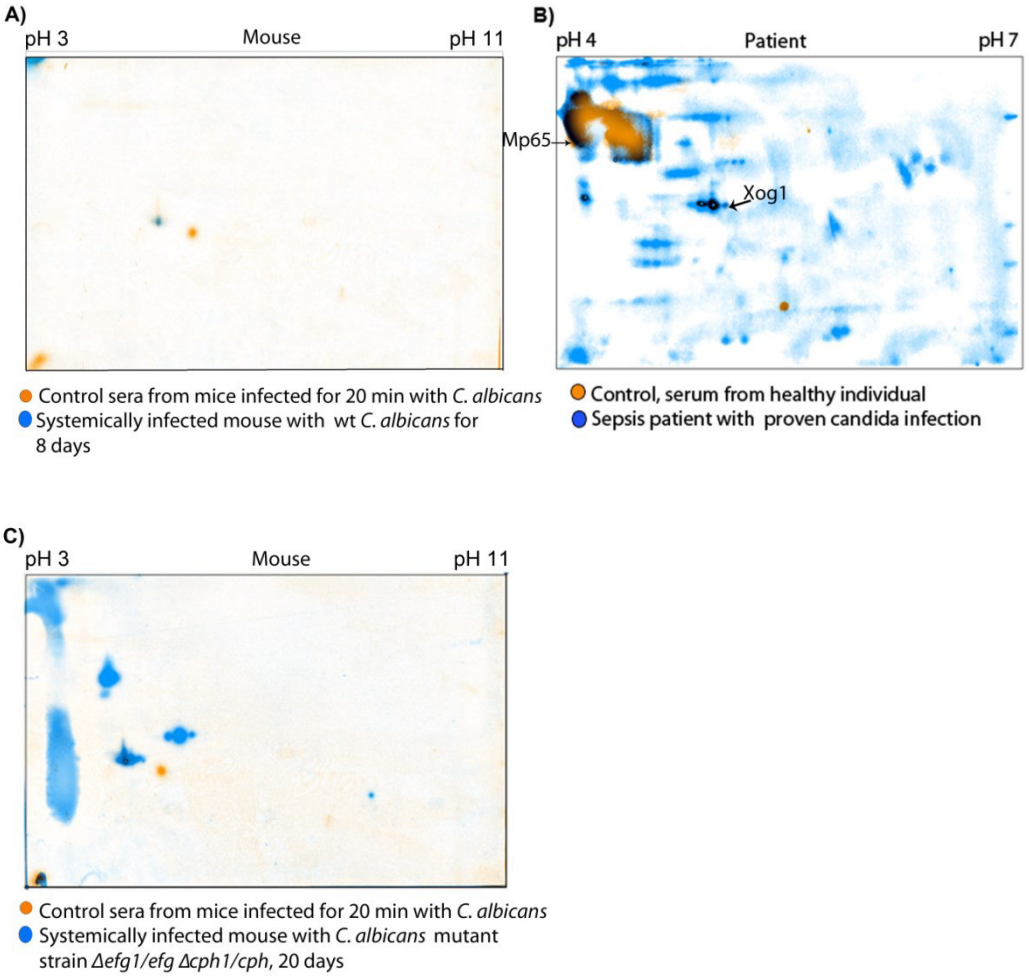
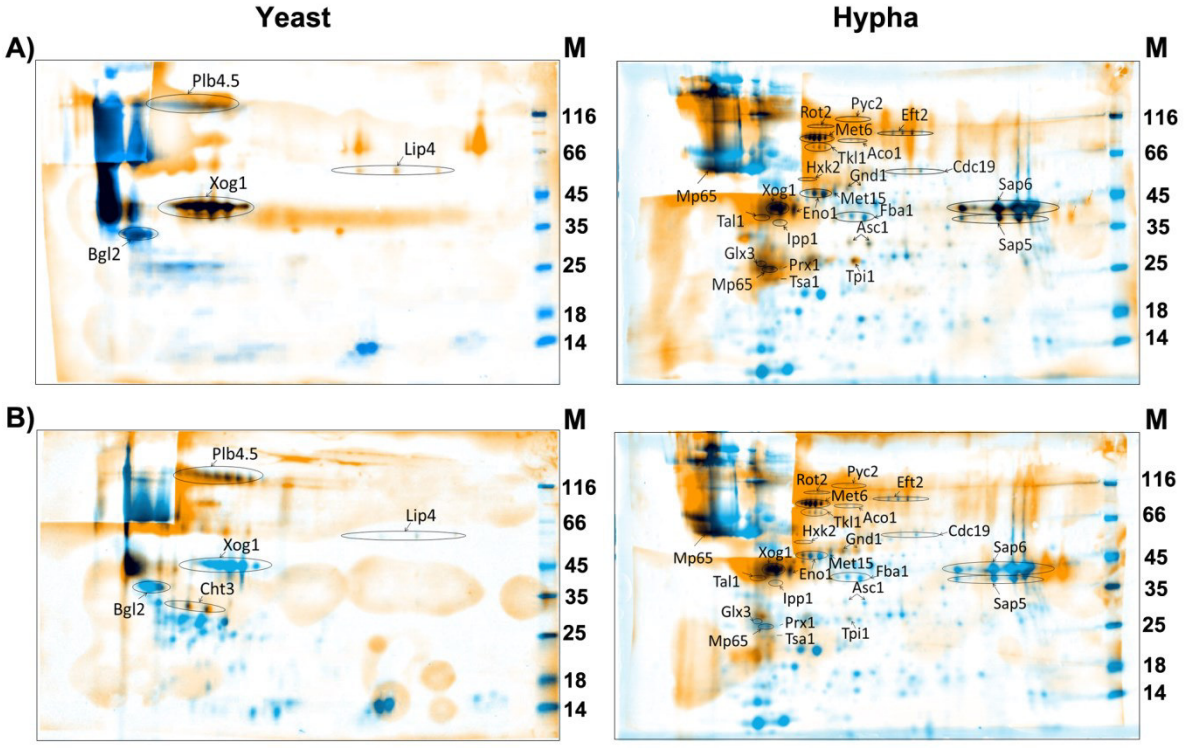


Figure14 Exemplary 2D immunoblot overlays for the comparison of the serological response of mice and candidemia patients to the *C. albicans* yeast secretome. A: 2D immunoblot overlays for the serological response of mice to the *C. albicans* yeast secretome after 8 days infection in comparison to control mouse sera collected after 20 minutes infection with *C. albicans* wild type strain; B: Comparison of the serological response of mice systemically infected with *C. albicans* mutant strain Δ*efg1*/*efg* Δ*cph1*/*cph* with control mouse sera described in A); C: Comparison of the

serological response of candidemia patients to the *C. albicans* yeast secretome with control serum from healthy blood donor.

3.2 Mapping of the *C. albicans* immunosecretome in candidemia and non-candidemia patient groups

To investigate the human host-specific serological response to the *C. albicans* secretome, sera samples from candidemia patients were compared with sera samples from SIRS patients, bacteremia (without fungal infection) and patients without suspected infection by applying serological proteome analysis (SERPA), which combines 2D-gel electrophoresis with immunoblotting. Details about the sera samples used are listed in Table 4. In Figure 15, *C. albicans* yeast and hyphal 2-D immunoblot overlays are exemplarily shown, which demonstrate the differences in antigen recognition of secreted proteins by human IgGs from sera of the different patient groups described above.



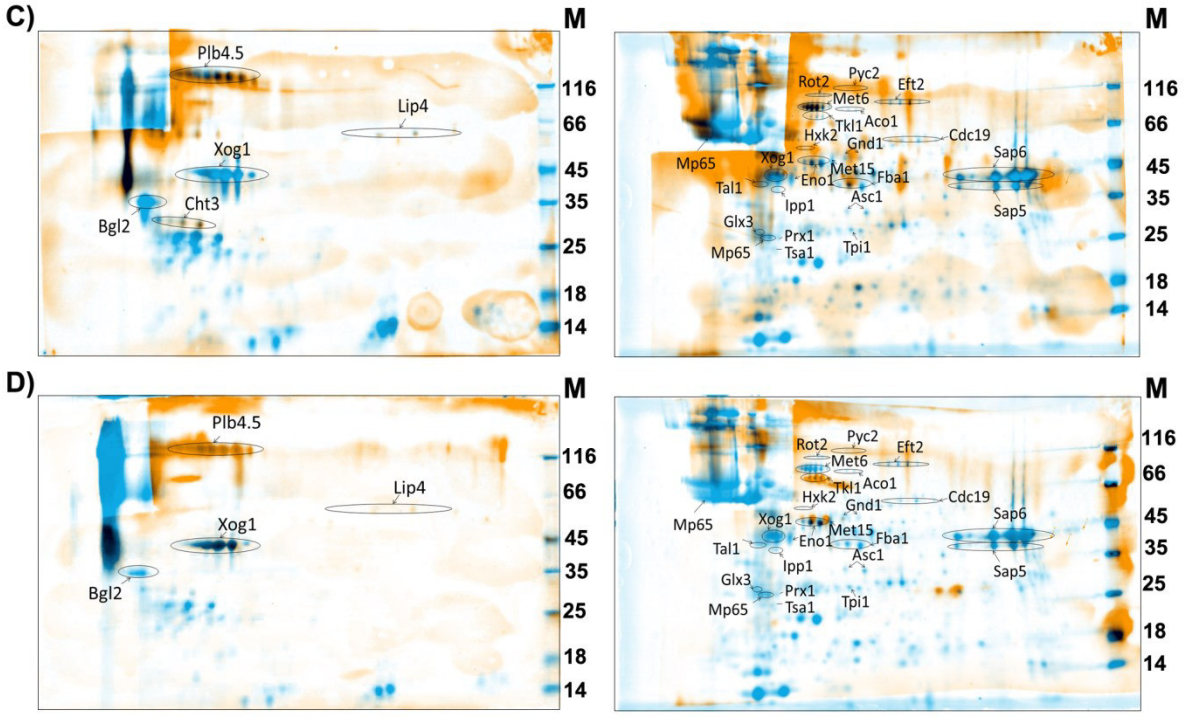


Figure 15 *C. albicans* yeast and hyphal secretomes recognized by serum IgG antibodies from candidemia and non-candidemia patients groups. 2-D immunoblot overlays show the differences in the *C.albicans* yeast and hyphal secretome antigen recognition profiles from representative patients of each sera sample group. A) Candidemia patient; B) SIRS patients; C) bacteremia patients; D) negative control (patients above 60 years without suspected infection). Blue-coloured images represent detected protein spots and orange-coloured images show Western blot signals from the anti-*C. albicans* antibodies in patients´ sera. The identified proteins are indicated.

3.3 Sera IgG titer assay of candidemia and non-candidemia control groups in response to *C. albicans* hyphal secretome

The titers of sera IgGs directed against the *C. albicans* hyphal secretome in candidemia and non-candidemia patients were determined by ELISA. The median values of anti-*C. albicans* IgG titers in sera of sepsis patients were much higher than in the other control groups. Nevertheless, the highest anti-*C. albicans* IgG titer in the control group of patients without suspected infection and in the SIRS group were as high as the level observed in sepsis patients. The lowest anti-*C. albicans* IgG titer in the sera of sepsis patients was as low as the level determined in healthy, voluntary donors (Figure 16A). Figure 16B shows that the median values of anti-*C. albicans* IgG titers in candiduria patients and candidemia patients with other than *C. alibcans*

species infection were only a little lower than in candidemia patients with only *C. albicans* infection. Candiduria patients were diagnosed by the detection of *C. albicans* in the urine culture. The anti-*C. albicans* IgG titer fluctuated considerably in the sera of sepsis patients with both bacterial infection and candida infection (Blood F+B) as well as SIRS patients with acute pancreatitis (SIRS A.P.).

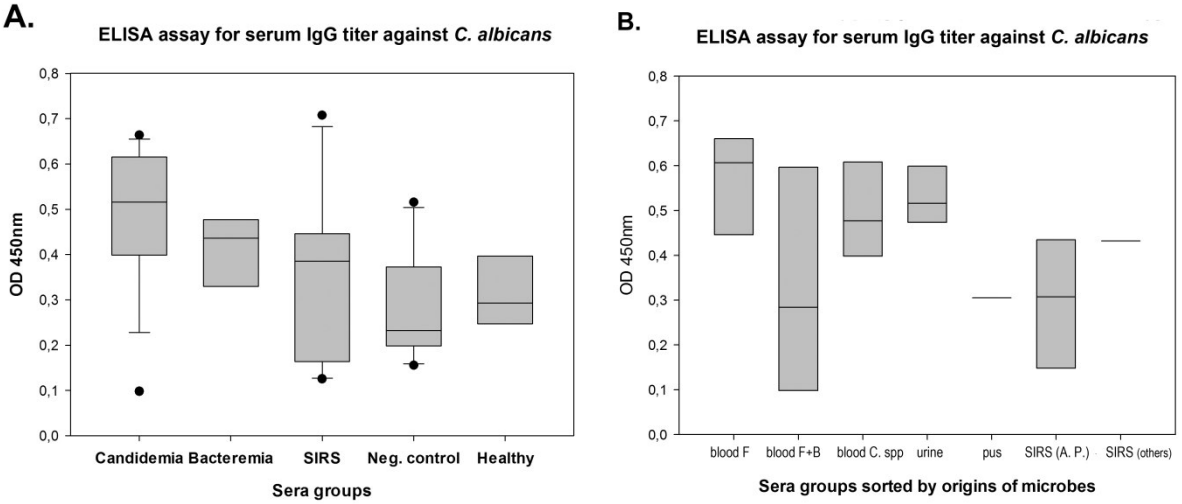


Figure 16 ELISA assay of the anti-*C. albicans* IgG titer in sera of candidemia and non-candidemia patients. Blood F: sera samples positive for *C. albicans* in the blood culture; Blood F+B: sera samples positive for *C. albicans* and bacteria positive in the blood culture; Blood *C. spp*: sera samples positive for *C. spp.* in the blood culture; Urine: urine samples positive for *C. albicans*; Pus: *C. albicans* were detected in the pus samples; SIRS (A.P.): acute pancreatitis patients without suspected systemic infection

3.4 Serological profiles of IgG antibodies directed against *C. albicans* secreted proteins

Despite the high heterogeneity of the protein antigen recognition patterns by patient IgG antibodies, 20 different protein antigens were frequently detected by sera of candidemia patients. A total of two yeast-specific and 17 hyphal-specific secreted proteins were identified as immunoreactive in at least four out of five patients with candidemia. However, they all showed serum-positive antigenicities in other non-candidemia patients groups as well (Table 8). In addition, one secreted protein, the β-glucanase Xog1p, recognized by sera in the secretomes of hyphal *C. albicans* cells, was also recognized by three serum samples out of five candidemia patients in the yeast secretome. The protein antigens´ functions were mainly associated with

stress responses (G-beta-like protein Asc1p, Elongation Factor 2 Eft2p, likely cobalamin-independent methionine synthase Met6p, putative vacuolar protease B Prb12p and two likely thioredoxin peroxidases Prx1p and Tsa1p), carbohydrate metabolism (pyruvate kinase Cdc19p, triose-phosphate isomerase Tpi1p, enolase Eno1p, fructose-bisphosphate aldolase Fba1p, aconitase Aco1p, putative pyruvate carboxylase Pyc2p, transaldolase Tal1p, putative transketolase Tkl1p, alpha-glucosidase Rot2p and exo-1,3-beta-glucanase Xog1p) and lipid degradation (lipase Lip4p and phospholipase B Plb4.5p) (Table 8).

Table 8 Analysis of the IgG antibody response against *C. albicans* extracellular proteins in candidemia patients and control groups.

Identified *C. albicans* antigens				Serum IgG response to *C. albicans* proteins No. of seropositive patients				Ab. response reported in	
Name	**CGD accession**	**Yeast Ag.**	**Hyphal Ag.**	**Candidemia (n= 5)**	**SIRS (n=5)**	**Bacteremia (n=5)**	**Neg. control (n=6)**	**mice**	**human**
Reponse to stress									
Asc1	orf19.6906		√	4	1	2	2	/	Y^{12}
Eft2	orf19.5788		√	5	5	5	4	$Y^{12;13}$	$Y^{12;13}$
Met6	orf19.2551		√	5	4	2	0	$Y^{13;17;18}$	$Y^{13;17;18}$
Prb12	orf19.7196		√	5	5	3	3	/	/
Prx1	orf19.5180		√	4	1	1	3	/	/
Tsa1	orf19.7417		√	4	2	0	3	$Y^{13;15}$	/
Proteases and lipases									
Lip4	orf19.2133	√		5	3	5	4	/	/
Plb4.5	orf19.1442	√		5	5	5	6	/	/
Carbohydrate metabolic process									
Cdc19	orf19.3575		√	4	2	2	3	$Y^{11;13;15}$	/
Tpi1	orf19.6745		√	4	2	1	2	Y^{11-15}	Y^{11-15}
Eno1	orf19.395		√	5	4	4	3	Y^{14}	Y^{16}
Fba1	orf19.4618		√	5	1	3	2	Y^{11-13}	Y^{11-13}
Aco1	orf19.6385		√	4	4	1	1	$Y^{11;12}$	$Y^{11;12}$
Pyc2	orf19.789		√	5	5	5	4	/	/
Tal1	orf19.4371		√	4	4	3	3	/	/
Tkl1	orf19.5112		√	5	5	4	6	$Y^{12;13}$	$Y^{12;13}$
Rot2	orf19.974		√	5	5	3	4	/	/
Xog1	orf19.2990		√	5^{H}; 3^{Y}	5^{H}; 2^{Y}	3^{H}; 3^{Y}	6^{H}; 5^{Y}	/	/
Unknown function									
Ssp120	orf19.3173		√	4	2	1	0	/	/

Superscript numbers in the table refer to references that reported the antibody response to specific *C. albicans* proteins in mice or humans. Superscript H stands for hypha; Superscript Y stands for yeast.

3.5 Glycosylation of secreted proteins has a significant effect on the antigen recognition by IgG antibodies

3.5.1 Glycosecretome

To investigate whether *C. albicans* secreted hyphal proteins are modified by glycosylation, the periodic acid-schiff (PAS) method was applied for the staining of *C. albicans* extracellular glycoproteins. Mp65p and Sap6p turned out to be the most heavily glycosylated proteins in the *C. albicans* hyphal secretome (Figure 17).

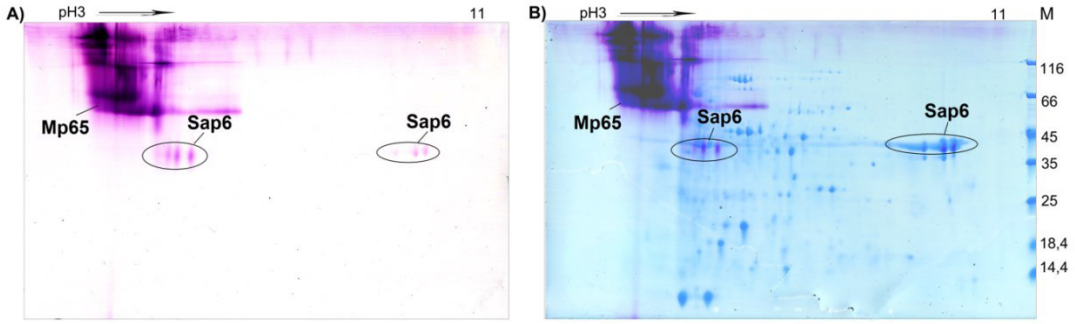


Figure 17 PAS-glycostaining of 2-D hyphal secretome gel (A) was followed by Coomassie staining (B) for total protein detection.

3.5.2 Dependence of the immunoreactivity of Sap6p on the glycosylation status

In order to investigate whether glycosylation plays a critical role in the recognition of protein antigens by IgG antibodies, oligosaccharide residues were removed from extracellular proteins by enzymatic treatment using a commercial deglycosylation kit. Figure 18A and 18C illustrate that deglycosylation leads to changes of the isoelectric point and molecular mass of several proteins. The anti-Sap6p antibody signal was drastically reduced after deglycosylation (Figure 18B and 18D). This indicates that sugar residues of Sap6p are major part of the epitope that is recognized by patient's anti-Sap6p antibodies. Moreover, also Mp65p and other cell wall proteins in the upper-left corner showed reduced antibody levels in candidemia patients after deglycosylation.

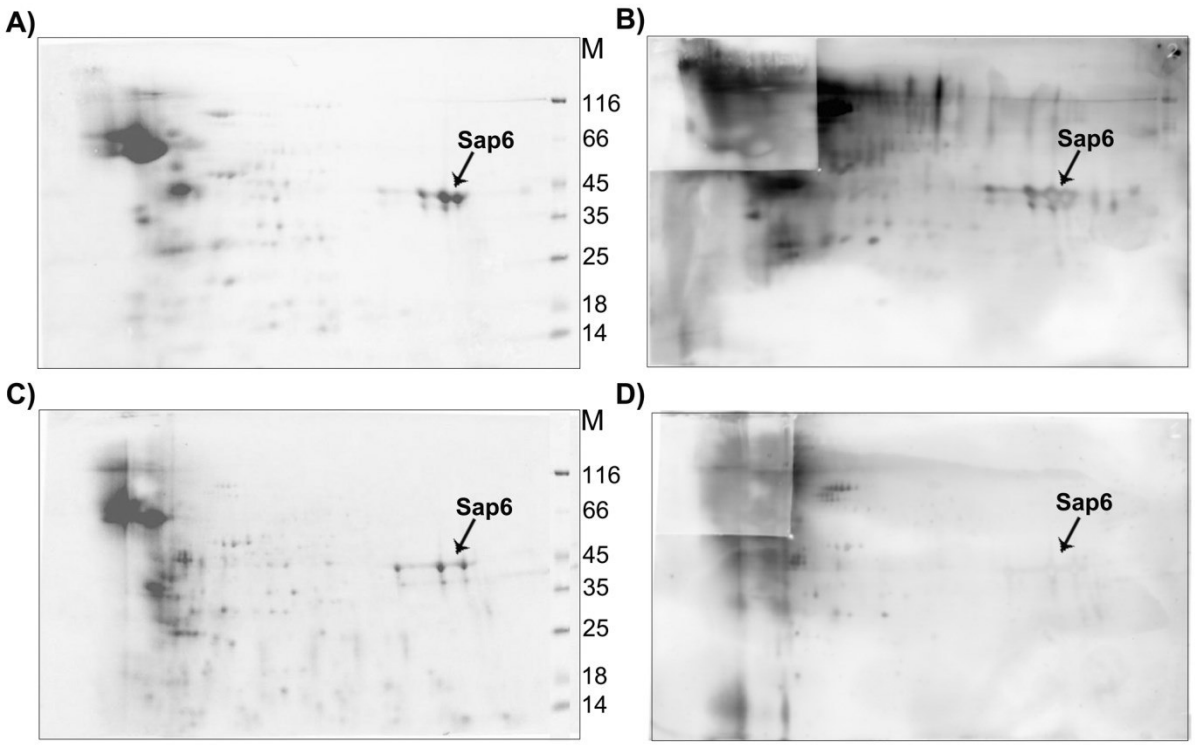


Figure 18 Immunoreactivity of Sap6p in sera of candidemia patients after deglycosylation. 2D membrane images of an untreated *C. albicans* hyphal secretome sample (A) and a deglycosylated *C. albicans* hyphal secretome sample (C); B) and D) show the corresponding Western blot signals of IgG serum antibodies from one representative candidemia patient, respectively. In the top left-hand corner of B) and D), the excised left corners represent mainly the patient sera antibody response against *C. albicans* mannoprotein Mp65p.

3.5.3 Glycosylation of recombinant Sap6p

To investigate the importance of Sap6p glycosylation for antigen recognition in more detail, we used a recombinantly produced Sap6p that had been heterologously expressed in *Pichia pastoris*. In Figure 19B recombinant Sap6p was separated in four bands after SDS-PAGE (ca. 62 kD, 40 kD, 30 kD and 10 kD). These four bands were all identified as Sap6p by MALDI-TOF/TOF. The smaller bands of 30 kD and 10 kD most likely represent self-proteolytic products of Sap6p (40 kD). The Sap6p protein bands with a respective molecular mass of 62 kD, 40 kD and 30 kD were differentially glycosylated (Figure 19A). The degree of glycosylation decreased apparently from the 62 kD band to 30 kD band. After deglycosylation, the apparent molecular masses of the 40 kD and 30 kD bands of Sap6p decreased. By contrast, no shift was observed for the 62 kD band after deglycosylation (Figure 19A

and B). The 62 kD band may represent a dimer due to incomplete denaturation. Its steric conformation may prevent enzymatic deglycosylation.

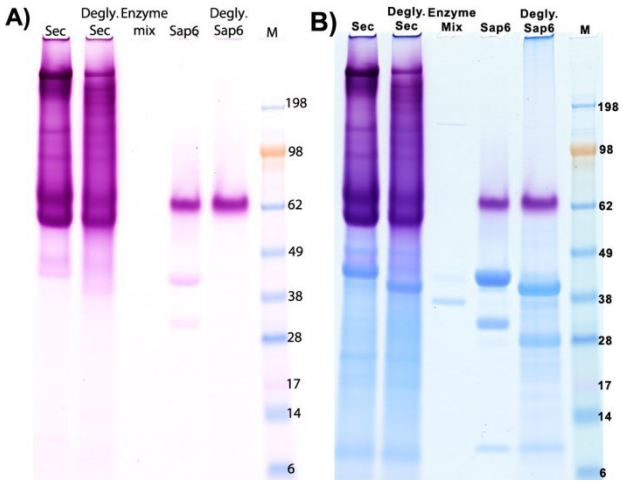


Figure 19 Identification of protein glycosylation in the secretome of *C. albicans* hyphal cells and the recombinant Sap6p protein A) Staining of the *C. albicans* secretome of hyphal cells and the recombinant Sap6p protein (expressed in *P. pastoris*) based on the periodic acid-Schiff (PAS) reaction. B) Post-staining of the gel shown in A) with Coomassie Blue for the global visualization of separated protein bands (comparison of glycoproteins with non-glycosylated proteins).

3.5.4 Immunoreactivity of recombinant Sap6p in sera of candidemia patients after deglycosylation of Sap6p

Although the serum antibody response to recombinant Sap6p showed a highly interindividual variety among the patients´ groups, deglycosylated recombinant Sap6p proteins were hardly recognized by anti-Sap6p serum antibodies from all sera species, including four candidemia, two SIRS, two bacteremia patients and four negative controls (two patients without infection and two healthy blood donors) (Figure 20). Notably, after deglycosylation, the intensity and molecular mass of the 62 kD band did not change. Altogether our experiments with the recombinant Sap6p confirmed that glycosylation of Sap6p plays an important role in its recognition by anti-Sap6 antibodies.

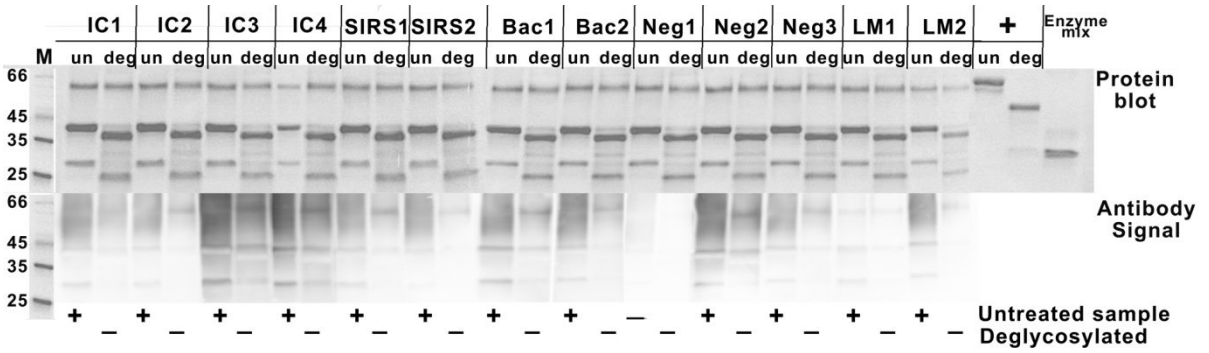


Figure 20 Comparative analysis of the recognition of the recombinant *C. albicans* Sap6p antigen by serum antibodies from candidemia and non-candidemia patients before and after deglycosylation treatment

IC: invasive candidiasis; SIRS: systemic inflammatory syndrome; Bac: bacteremia; Neg.: negative control; LM: healthy donor (lab member); un: untreated sample; deg: deglycosylated sample; M: molecular mass marker proteins

Discussion

During yeast-to-hypha transition *Candida albicans* secretes numerous proteins for nutrition acquisition, tissue damage and modulation of the immune response (Naglik *et al.* 2004; Schaller *et al.* 2005). The aim of the present work was to investigate the dynamics of the *C. albicans* secretome composition during the yeast-to-hypha transition as well as its ability to elicit serological responses in candidemia patients.

Several studies have analyzed the global protein composition of the *C. albicans* secretome including the content of secreted extracellular vesicles under different growth conditions by LC-MS/MS (Sorgo *et al.* 2010; Sorgo *et al.* 2011; Ene *et al.* 2012; Gil-Bona *et al.* 2015). Here, we characterized the secretomes of yeast and hyphal cells of *C. albicans* by combining two proteomic approaches, *i.e.*, 2D-gel electrophoresis and mass spectrometry-based proteomics, to deliver a comprehensive picture of the set of secreted proteins. Furthermore, an immunoproteomic approach was applied to identify *C. albicans* extracellular protein antigens, which provoke a potent antibody response in patients with candidemia.

1 Qualitative analysis of *C. albicans* yeast and hyphal secretome during the yeast-to-hypha transition

1.1 Comparison of gel-based with gel-free proteomic approach

By combining two proteomic approaches, 101 different secreted yeast and 410 hyphal proteins were identified (Table S-1). Several secreted proteins were detected in the culture supernatant for the first time, like some cell wall proteins (Cdc3p, Exg2p, Hyr1p and Mnt2p), secreted proteases (Ape3p, Cpy1p), adhesin (Als1p) and lipases (Lip4p and Plb2p). The majority of the detected proteins were identified by LC-MS/MS (92 % of identified yeast proteins and 95 % of hyphal proteins), whereas 8 yeast and 19 hyphal proteins were exclusively detected by 2D gel electrophoresis. This may be explained by the technical limitation of 2D gel electrophoresis to separate hydrophobic, low abundant, small and high molecular mass proteins and proteins with extreme p*I* (Aebersold *et al.* 2003; Speers *et al.* 2007; de Godoy *et al.* 2008; Kniemeyer *et al.* 2011). However 2D-GE provides an overview about the

features of individual, intact proteins including PTMs. It is particularly suitable for antigen identification by combining 2D-GE with immunoblotting.

1.2 Classical secreted proteases, lipases and adhesins

During yeast-to-hypha transition the *C. albicans* secretome undergoes significant dynamic changes. We found that some members of the Sap family, agglutinin-like sequence (ALS) family or lipases were detected either exclusively in the supernatant of yeast or hyphal cells (Table 11). It is well known, that the pH strongly affects expression of different *SAP* genes (Remold *et al.* 1968; Ruchel 1981; Morrison *et al.* 1993; White *et al.* 1993; Smolenski *et al.* 1997; Borg-von Zepelin *et al.* 1998). Indeed, we detected Sap2p, Sap3p and Sap10p, whose functional activities are optimal at pH3-5, exclusively in yeast cell cultures. By contrast, the hypha-associated proteases Sap4p, Sap5p and Sap6p were reported to be active at a higher pH of 5-7 (Naglik *et al.* 2003), and accordingly they were only detected in the medium supernatant of hyphal cells.

Table 11 Summary of identified lytic enzymes and proteins involved in cell wall organization

	Secreted proteases	**Adhesins**	**Lipases**	**Chitinases**	**Cell wall organization**
Yeast specific	Sap2, Sap3, Sap10	Als2, Als4	Lip4	Cht1	Phr2, Pir1, Ssu81, Sur7, Orf19.6741
Hyphae specific	Sap4, Sap5, Sap6	Als1, Als3			Abg1, Act1, Kre5, Mnt2, Phr1, Pmi1, Rbt1, Sod1, Tsa1, Orf19.2677
Common in yeast and hypha	Sap7, Sap9		Plb4.5	Cht2, Cht3	Bgl2, Crh11, Ecm33, Kex2, Mnt1, Msb2, Pga4, Rhd3, Rho1, Sim1, Sun41, Utr2, Xog1, Yps7

1.3 Cell wall-associated proteins

Many cell wall proteins were detected in the *C. albicans* yeast and hyphal secretome. This result is also consistent with Sorgo´s study (2010), which found approximately 57 % of detected secreted proteins to be cell wall-related (Sorgo *et al.*

2010). This finding suggests that cell wall-bound proteins are partially released from the fungal cell walls during growth. In this study, more than half of the identified proteins, which are involved in cell wall organization, were found in both culture supernatants of yeast cells and hyphae. These proteins include chitinases (Cht2p, Cht3p), chitin transglycosylases (Crh11p, Utr2p), glucanosyltransferases (Bgl2p, Pga4p), glucanases (Xog1p, Sim1p and Sun41p) and lytic enzyme (Sap9p) (Table 11). Also, many proteins exclusively detected in yeast or hyphal cell walls were found in this study, like the yeast-associated proteins Phr2p and Pir1p as well as the hypha-associated proteins Act1p, Kre5p, Phr1p, Rbt1p, Sod1p and Tsa1p. Based on GO-Slim annotation (*Candida* Genome database), these hypha-associated proteins are mainly involved in the response to alkaline environments, like Phr1p, Rbt1p and Sod1p, oxidative stress, such as Act1p, Sod1p and Tsa1p, or contribute to virulence, for example Kre5p, Mnt2p, Phr1p, Rbt1p and Sod1p. This suggests that hypha cells, in contrast to yeast cells, are likely under alkaline pH and oxidative stress conditions and become invasive to host cells. Hyphal cell wall proteins presented in the medium supernatant are highly regulated by these stress stimuli and correlate with the pathogenic program. This could also be explained by some cell wall proteins detected in this study, such as Sod1p, Rbt1p and Phr1p, which are inducible by the stress stimuli and also involved in virulence.

1.4 Non-conventional secretory proteins

Notably, only 107 out of 446 secreted proteins carry an *in silico* predicted signal peptide for secretion. A large number of non-canonical secretory proteins (339) were present in the medium supernatant, particularly of hyphal cells. These cytosolic proteins lacking a secretion signal are mostly associated with carbohydrate metabolism, response to stress and translation. They may represent ’moonlighting’ (multifunctional) proteins as previous secretome studies of *Saccharomyces cerevisiae*, *C. albicans* and several bacteria postulated (Nombela *et al.* 2006; Gil-Bona *et al.* 2015; Gotz *et al.* 2015). Notably, 6 and 15 proteins mediating vesicle-transport were detected in yeast and hyphal secretomes, respectively, which indicates that membrane vesicles may drive this unconventional protein secretion. In support of these findings, Ana *et al.* and Gabriele *et al.* recently reported that cytoplasmic proteins of *C. albicans* are transported *via* extracellular vesicles to the

outside (Gil-Bona *et al.* 2015; Vargas *et al.* 2015). It was shown that Eno1p, Rho1p, Tdh3p, Pdc11p, and Ypt31p were present in extracellular vesicles of *C. albicans* yeast cells. These proteins were also detected in this present study. Besides, Sorgo found that most cytosolic proteins were only detected in the supernatant of hyphal- and fluconazole-treated cells (Sorgo *et al.* 2011), which indicates that the export of proteins without obvious signal sequence is highly regulated by environmental stimuli. Alternatively, they could have been released due to the lysed cells within a natural population of cells.

In summary, the basic composition of *C. albicans* proteins, contributing to the maintenance of the cell wall, is similar in yeast and hypha. By contrast, extracellular proteins connected to stress responses differ substantially between the two morphotypes.

2 Quantitative analysis of *C. albicans* yeast and hyphal secretomes

The quantitative analysis of *C. albicans* yeast and hyphal secretomes *via* TMT labeling has shown that only 7 proteins are two-fold more abundant in yeast than in hyphae, whereas 155 proteins are two-times enriched in hyphal cells compared with yeast cells. This indicated that *C. albicans* cells secrete significantly more proteins with high abundance during the yeast-to-hypha transition. This result is also consistent with the qualitative analysis of the *C. albicans* secretome. The detected proteins, which were shared by both yeast and hyphal cells, are mainly cell wall proteins, proteases, adhesins, chitinases and lipases. Notably, several proteins, which were identified only in one morphotype in the qualitative analysis of secretomes, are still detectable in the quantitative analysis with TMT labeling. For example, Pir1p protein is a basic cell wall structure protein of *C. albicans*. It has been reported that the expression of *PIR*1 is repressed by Rim101p and downregulated in hyphae (Sohn *et al.* 2003; Lotz *et al.* 2004). In the quantitative analysis shown here, Pir1 was detected as a protein with higher abundance in yeast cell than in hypha, whereas it was only detectable in yeast but not hypha in our qualitative study. The explanation for that could be, that some morphologically regulated proteins are just enriched in one morphotype and very low abundant in the other (maybe below

detection limit). To quantify the relative protein abundance, the TMT-labeling approach is more reliable than a label-free approach using spectral counting.

3 Peptide analysis in the *Candida* hyphal secretome

Besides intact extracellular proteins, intracellularly proteolytic processed peptides are also detectable in the culture supernatant of *C. albicans* hyphal cells. Here, it was demonstrated that Ece1p was the most abundant processed protein among all SPE-enriched peptides. This finding is also in line with the transcriptional expression of *C. albicans* hyphal cells, which indicated that *ECE*1 was highly expressed during hyphal growth (Birse *et al.* 1993). It is known that Ece1p is processed by the protease Kex2p into eight peptides (Bader *et al.* 2008). Among them, the peptide III turned out to be the most dominant peptide in the culture supernatant based on the LC-MS/MS analysis. The *C. albicans* peptide Ece1p-III has recently been shown to represent a cytolytic peptide toxin, the first described peptide toxin in a human pathogenic fungus (unpublished data, Moyes *et al.*). It is only produced by the invasive hyphal form of *C. albicans*. The Ece1p-III peptide is an amphipathic molecule. It has a positively charged C-terminus, which is required for membrane permeability and triggers an inward current concomitant with calcium influx. It possesses also a hydrophobic transmembrane domain with an α-helical structure for cytolysis of multiple human epithelial cell types. It was demonstrated that the Ece1p-III peptide directly damages epithelial membranes, by triggering p-MKP1/c-Fos mediated danger response pathway and secreting the anti-inflammatory cytokines for activating epithelial immunity (unpublished data, Moyes *et al.*).

4 Serological response of mice to *C. albicans* yeast secreted proteins

Although *C. albicans* is not a commensal on the mucosal surface of mice, mouse models of invasive *C. albicans* infection by intravenous injection are still the most popular method to assess *Candida* virulence until today (Maccallum 2012). Besides determining virulence, mice models of systemic candidiasis allow also characterizing the serological response to *C. albicans*. One of the results of studying the serological response in mice, which are shown here, is that the generation of *C. albicans*-specific IgG antibodies takes at least five days upon infection. Secondly, the serological responses to secreted proteins of *C. albicans* yeast cells during systemic

infections are less pronounced and diverse in comparison to humans. This may be explained by the fact, that *C. albicans*, in contrast to mice, is a normal constituent of the human flora, which leads to a basal anti-*C. albicans* antibody level in human sera. The second reason for the differences may lay in the fact that mouse antibody IgG subclasses differ from human IgG subclasses, which might cause different antibody responses (Mestas *et al.* 2004). Thirdly, human sera may contain more cross-reacting IgG antibodies due to complex disease background and a heterogenous gastrointestinal flora, which could induce the production of cross-reactive antibodies that recognize *C. albicans* antigens.

5 Serological profiles of human IgG antibodies directed against *C. albicans* secreted proteins

As shown here, IgG antibodies directed against *C. albicans* are generally detectable in human sera, albeit showing weak and delayed antibody responses in some patients. To get a deeper insight into the antibody response against secreted proteins of *C. albicans*, an immunosecretome reference map of *C. albicans* was established by screening sera of systemic candidemia patients using a gel-based proteomic approach.

5.1 Comparison of *C. albicans* yeast immunosecretome with hyphal immunosecretome in candidemia patients

Altogether, two yeast and 17 hyphal secreted immunogenic proteins were identified. This finding implied that hyphal cells elicited a stronger antibody response than yeast cells possibly due to the fact, that the hypha is the invasive morphotype of *C. albicans* (Gow *et al.* 2012) (Table 8). This finding fits well with many other *in vitro* and *in vivo* studies which showed that the hyphal form of *C. albicans* invades epithelial and endothelial cells and causes damage, in particular by the release of hydrolytic enzymes and toxic peptide (Scherwitz 1982; Filler *et al.* 2006; Phan *et al.* 2007; Dalle *et al.* 2010; Zhu *et al.* 2010; Sudbery 2011; unpublished data, Moyes *et al.*). The immunogenic proteins we identified mainly included proteins in response to

oxidative stress, starvation stress, heat and drug stress (Asc1p, Eft2p, Met6p, Prb12p, Prx1p and Tsa1p), lytic enzymes (Lip4p and Plb4.5p), cell wall polysaccharide and glucose metabolism-associated proteins (Cdc19p, Tpi1p, Eno1p, Fba1p, Aco1p, Pyc2p, Tal1p, Tkl1p, Rot2p and Xog1p). Among them, 9 antigens have already been reported by Pitarch *et al.* (Pitarch *et al.* 2004). They all represent abundant housekeeping proteins (Aco1p, Asc1p, Eft2p, Eno1p, Fba1p, Met6p, Tkl1p, Tpi1p and Tsa1p), localized in the cytoplasm, but all are also detectable in the extracellular space. This finding indicates that these housekeeping enzymes also play a role outside the cell wall and interact with the host immune cells.

In this study, nine proteins (Prb12p, Prx1p, Lip4p, Plb4.5p, Pyc2p, Tal1p, Rot2p, Xog1p and Ssp120p) have been described to be immunogenic for the first time. Intriguingly, *C. albicans* yeast cells secreted two lipases, e.g., lipase Lip4p and phospholipase Plb4.5p, triggered antibody production during systemic candidiasis. They both represent extracellular lipolytic enzymes, which may contribute to host tissue invasion by degrading host surface molecules and destroying cell membranes. It has been reported that *C. albicans* phospholipase B is secreted to a higher amount by a *C. albicans* strain isolated from human blood than from a commensal strain (Ibrahim *et al.* 1995; Schaller *et al.* 2005). Furthermore, the *LIP4* gene was reported to be expressed during the early phase of systemic liver infection in mice and in samples of patients with oral candidiasis (Stehr *et al.* 2004).

5.2 Comparison of serological response of candidemia patients and non-candidemia patient groups directed against *C. albicans* secretome

The anti-*C. albicans* antibody levels in candidemia patients are in most cases higher than in non-candidemia control groups (Figure 15). In line with this finding, ELISA tests for sera IgG titer against the *C. albicans* hyphal secretome showed that the median value of the IgG titer against *C. albicans* hyphal secretome was much higher in sepsis patients than in control patients. However within the patient groups the anti-*C. albicans* antibody levels differed strongly. Notably, patients with a positive detection of *C. albicans* in urine samples also showed a strong serological response against secreted proteins of *C. albicans* as observed for candidemia patients. This

suggests that for the diagnosis of systemic candidiasis not only blood cultures, but also urine samples may be an additional indicative tool.

5.3 Serum levels of specific anti-*C. albicans* antibodies help to diagnose systemic candidiasis

The results reported here give ample reason to assume that there is no clear, unambiguous picture of an anti-*C. albicans* antibody pattern in candidemia patients. Since *C. albicans* is a commensal in most humans and surface infections occur frequently, a basal level of anti-*C. albicans* antibodies is present in most individuals. However, seven *C. albicans* extracellular antigens (Xog1p, Rot2p, Eno1p, Met6p, Tsa1p, Tpi1p and Prx1p) contribute to distinguish candidemia from bacteremia, SIRS and control patients. The mean level of the serological antibody response to each of these seven antigens is at least two fold higher in candidemia patients than in the other three control groups. For this reason, they may represent promising biomarkers for the diagnosis and prognosis of candidemia. Interestingly, the top 7 serodominant antigens of *C. albicans* (Xog1p, Rot2p, Eno1p, Met6p, Plb4.5p, Tkl1p and Tal1p) were also recognized by sera of SIRS and bacteremia patients (Table 12). Less immunoreactive proteins (antigens with low level of detection signals on immunoblot) showed, however, often less or no cross-reaction with sera of control groups (Table 12). Presumably, an unspecific epitope, which is shared by many immunogenic proteins, triggers antibody responses in patients with systemic inflammation including candidemia, SIRS and bacteremia. A common feature of these seven serodominant antigens is that they are localized in the extracellular space (based on their GO-Slim Mapper annotations categorized by the cellular components). It is well known that most *C. albicans* extracellular proteins are glycosylated (Delic *et al.* 2013; Hall *et al.* 2013). Furthermore polysaccharides are common dominant antigens on the cell surface of *C. albicans*, such as mannans and ß-glucans (Hasenclever *et al.* 1961; Chattaway *et al.* 1968; Poulain *et al.* 1985; Nelson *et al.* 1991). Therefore, these proteins most likely share glyco-epitopes or, alternatively, conserved amino acid sequences, which trigger antibody responses in patients with systemic inflammation.

Notably, anti-Plb4.5p antibody levels were consistently high in all patient groups, which indicate that the yeast-specific phospholipase Plb4.5p may represent a

highly abundant, secreted protein antigen of *C. albicans* yeast cells. The *C. albicans* secretome data also showed that Plb4.5p was among the top 5 abundant proteins in the yeast secretome when its peptide-spectrum matching (PSM) score was considered. Thus, Plb4.5p may confer a vital and basic biological function during host colonization, like nutrition acquisition by hydrolysis of ester linkages in glycerophospholipids from host tissues. In support of this finding, a *C. albicans* surface protein microarray showed that the mean antibody signal intensities to the top-forty serodominant antigens were slightly higher in healthy individuals than in non-infected hospital patients and acute candidemia patients (10,380 vs. 8,837 and 8,825, respectively) (Mochon *et al.* 2010). This suggests that a continuous interplay between the human host and *C. albicans* exists.

Table 12 Heat map of signal intensity of serum antibodies binding to the *C. albicans* secreted protein antigens. The colors red, orange, yellow, light green, dark green illustrate signal intensities ranging from very strong (red) to no signal (dark green). The antibody response signals were classified according to the grey intensity of spot signals in the class 3 (strong signal), 2 (middle), 1 (weak/no signal). Later, the average value of each antigen signal in each patient group was summarized.

Protein Name	Yeast antigens	Hyphal antigens	SP	Candidemia (n= 5)	SIRS (n=5)	Bacteremia (n=5)	Neg. control (n=6)
Xog1		√	√	3	1.6	1.6	1
Rot2		√	√	3	1.8	1.4	0.6
Eno1		√		2.8	1.4	1.4	1
Met6		√		2.8	1.8	0.8	0
Plb4.5	√		√	2.6	2.6	2.8	2.6
Tkl1		√		2.4	2.4	1.4	1.6
Tal1		√		2.4	2	0.6	1.2
Tsa1		√		2	0	0.4	0.5
Tpi1		√		2	0.4	0.2	0.5
Eft2		√		1.8	1.6	1.6	0.6
Pyc2		√		1.8	2.2	2.4	0.6
Prx1		√		1.6	0.2	0.2	0.5
Prb12		√	√	1.4	2.2	0.6	0.6
Lip4	√		√	1.4	0.6	1	0.8
Aco1		√		1.2	1.6	0.2	0.2
Cdc19		√		1	0.4	0.4	0.6
SSP120		√	√	1	0.4	0.2	0
Fba1		√		1	0.2	1	0.3
ASC1		√		0.8	0.2	0.4	0.4

5.4 Effect of glycosylation of secreted proteins on the antigen recognition

Glycoprotein staining of the *C. albicans* hyphal secretome showed that many secreted proteins were glycosylated. In particular the secreted protease Sap6p was highly glycosylated (Figure 17). This is in line with the fact that *C. albicans* Sap6p has been reported to be N-glycosylated (Aoki *et al.* 2011). *C. albicans* Sap6p was hardly recognized by serum antibodies after enzymatic removal of N- and O-glycans of hyphal secreted proteins of *C. albicans* or the recombinantly-produced Sap6p protein (Figure 18 and 19). It is therefore reasonable to conclude that the carbohydrate moiety of Sap6p represents a major epitope and is critical for triggering serological responses. A recent study strongly supports this finding. Hao *et al.* demonstrated that *C. albicans* Sap6p contains the adhesion motif RGD to bind integrins on epithelial cells, which results in the internalization of Sap6p to endosomes and lysosomes (Wu *et al.* 2013). Finally, this leads to the apoptosis of epithelial cells. Moreover, Sap6p was reported to be able to induce activation of the inflammasome in human monocytes and murine macrophages, which finally leads to innate immune cell activation (Pietrella *et al.* 2013; Gabrielli *et al.* 2015). Thus, it is conceivable, that Sap6p activity further mediates the adaptive immune response including the production of specific anti-Sap6p antibodies.

Intriguingly, serum anti-Sap6 antibodies were not only detected in candidemia patients, but also in sera of control groups including SIRS and bacteremia patients as well as patients without suspected infection. It is likely that the glyco-epitope of Sap6p is recognized by antibodies that are not specific for Sap6p. This glyco-epitope may share significant structural similarities with glyco-epitopes of other proteins or microorganisms. Secondly, although blood cultures in all non-candidemia control patients (SIRS, bacteremia and patients without suspected infection) were negative for *C. albicans*, a past episode of a transient or local invasive candidiasis in control patients cannot be excluded (Ostrosky-Zeichner 2012). In addition, an increased exposure to *C. albicans* due to translocation across the gut mucosa was observed in bacteremia and may also occur in SIRS patients (Otto *et al.* 2011).

Not only *C. albicans* Sap6p, but also many other secreted proteins showed considerably reduced sera antibody responses after deglycosylation treatment (Figure 18). For example, Mp65p, which is one of the most immunodominant antigens of *C. albicans*, was obviously much less immunoreactive after

deglycosylation (Figure 18B and 18D). However the serological antibody responses to some other protein antigens were not influenced after deglycosylation, which suggests the presence of peptide epitopes. These antigens mainly include abundant housekeeping proteins such as the serodominant antigens Met6p and Eno1p. In short, these findings implicate that glycosylation of extracellular proteins plays a critical role in the antibody response of *C. albicians*-infected patients. However, antibody responses directed to many cytosolic proteins are likely mediated by peptide epitopes. Furthermore, this study provides evidence that the serological response to glyco-epitopes of extracellular proteins allows distinguishing candidemia patients from negative controls despite the fact that glyco-epitopes was not species-specific and show cross-reactivity.

Conclusion

In this study, the secretome of *Candida albicans* during the yeast-to-hypha transition was qualitatively and quantitatively analyzed. The identified *C. albicans* secreted proteins mainly included cell wall-associated proteins, proteins in response to environmental stimuli, secreted proteases, lipases and adhesins as well as carbohydrate metabolism-associated proteins. In comparison to the yeast form, hyphal cells undergo cell wall remodeling and release significantly more proteins into the extracellular milieu. However, yeast cells share many common proteins with hyphal cells, which constitute the basic cell wall structure and function of *C. albicans* cells. Besides, intracellularly proteolytic processed, secreted peptides play an important role in the interplay with host cells. Peptide Ece1p III is the most abundant peptide in the supernatant of *C. albicans* hyphal culture. It has been identified as the first fungal cytolytic peptide toxin, which can directly damage epithelial cells, and consequently is crucial for mucosal invasion (unpublished data, Moyes *et al.*).

Extracellular proteins of *C. albicans* are accessible targets of the host immune system. The in-depth investigation of the serological response against secreted yeast and hyphal proteins of *C. albicans* in candidemia patients and three control groups (SIRS, bacteremia and patients without suspected infection) revealed a core set of 20 immunodominant anti-*C. albicans* secreted protein antibodies. Nine proteins (Prb12p, Prx1p, Lip4p, Plb4.5p, Pyc2p, Tal1p, Rot2p, Xog1p and Ssp120p) have been described to be immunogenic for the first time. Seven proteins represent potential diagnostic markers for candidemia (Xog1p, Rot2p, Eno1p, Met6p, Tsa1p, Tpi1p and Prx1p). Certainly, further studies are required for the validation of the determined antibody signatures.

Furthermore, glycosylation of *C. albicans* extracellular proteins not only enhanced the immunogenicity, but also induced unspecific binding of antibodies, leading to cross-reactivity of *C. albicans* antigens. Therefore, the identification of the immunogenic carbohydrate moiety, the so-called glyco-codes, which stimulates the antibody response in candidemia patients, could serve as a basis for the development of a new diagnostic assay or therapeutic vaccines.

Supplements

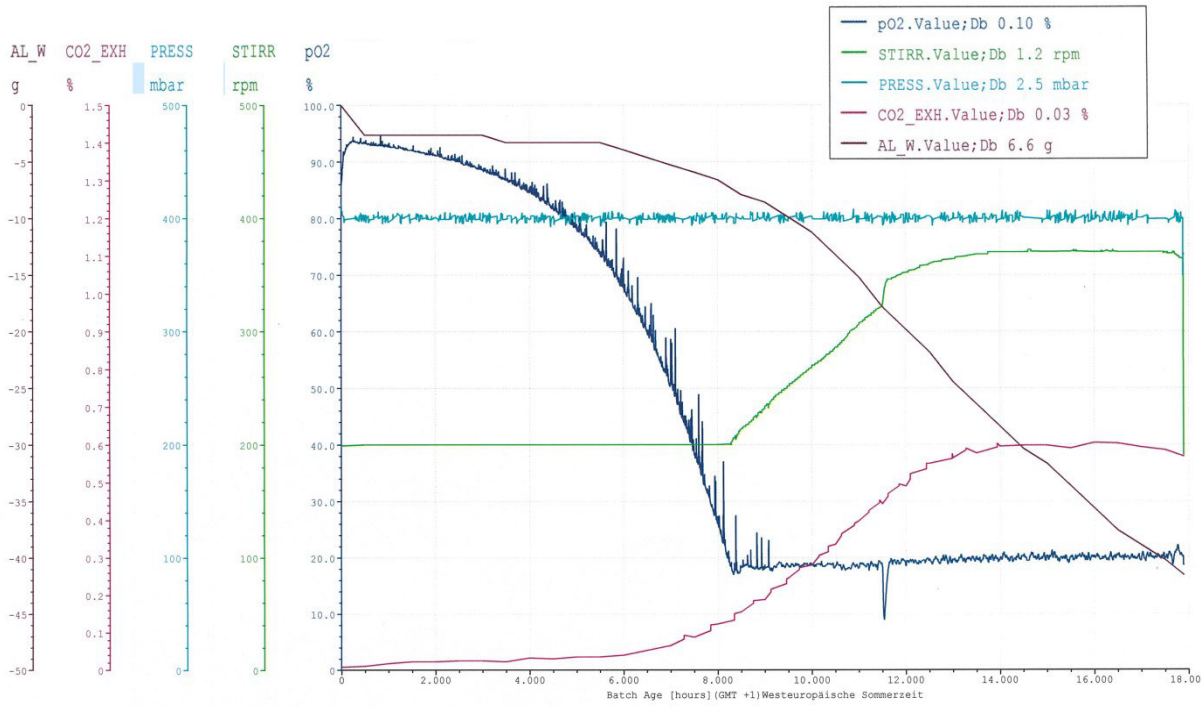


Figure S-1. Scatter plot of pO_2, stirring rate, pressure, CO_2 evolution and sodium hydroxide consumption during an 18 h cultivation of *C. albicans* yeast cells.

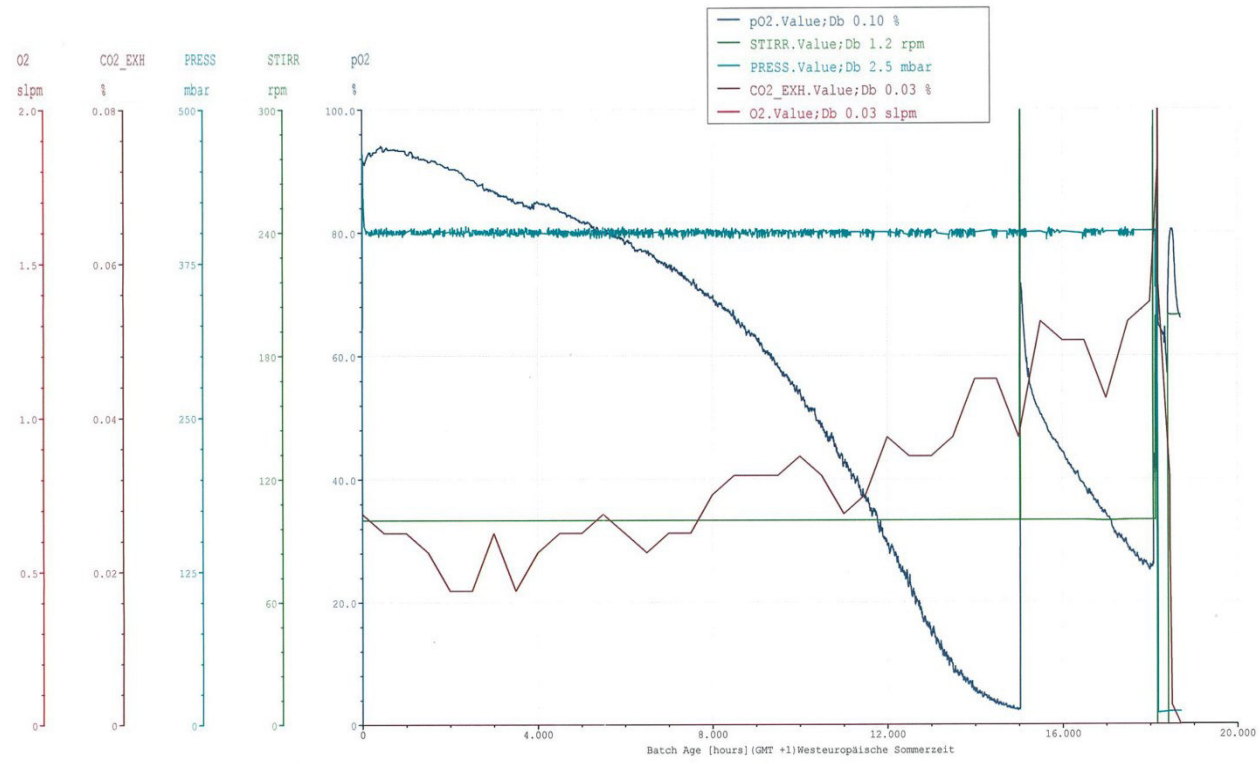
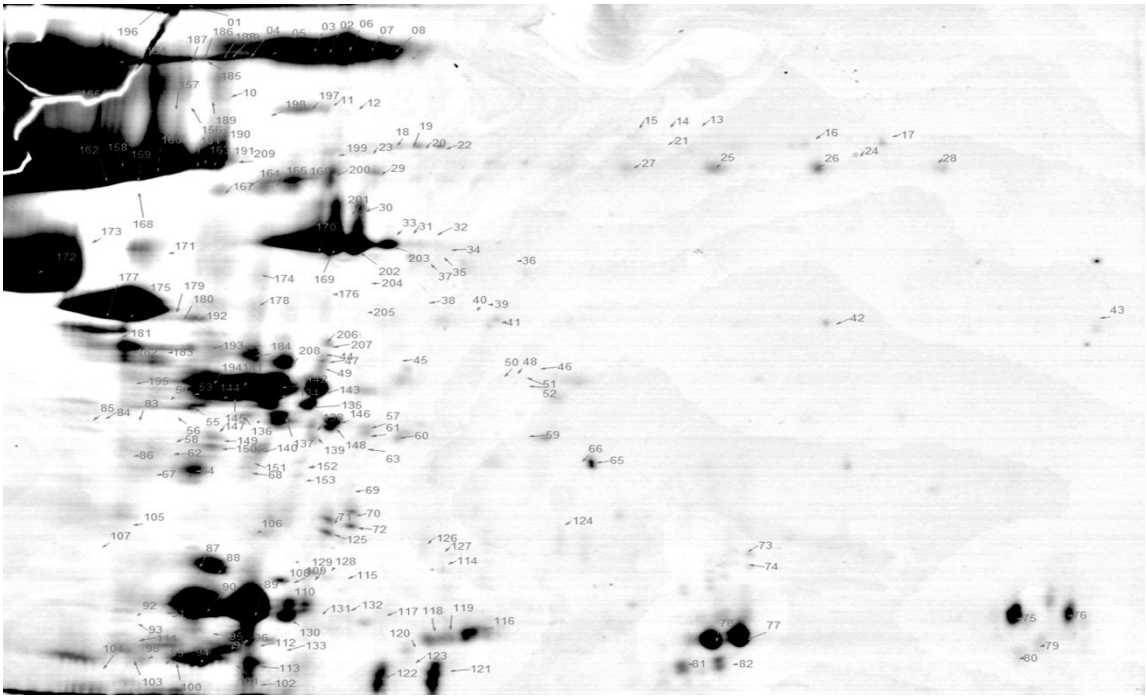


Figure S-2. Scatter plot of pO_2, stirring rate, pressure, CO_2 evolution and sodium hydroxide consumption during an 18 h cultivation of *C. albicans* hyphal cells.

A. *C. albicans* yeast secretome map pH4-7 with spot labeling



B. *C.albicans*hyphalsecretome map pH 4-7 with spot labeling

C. *C.albicans*hyphalsecretome map pH 7-11 with spot labeling

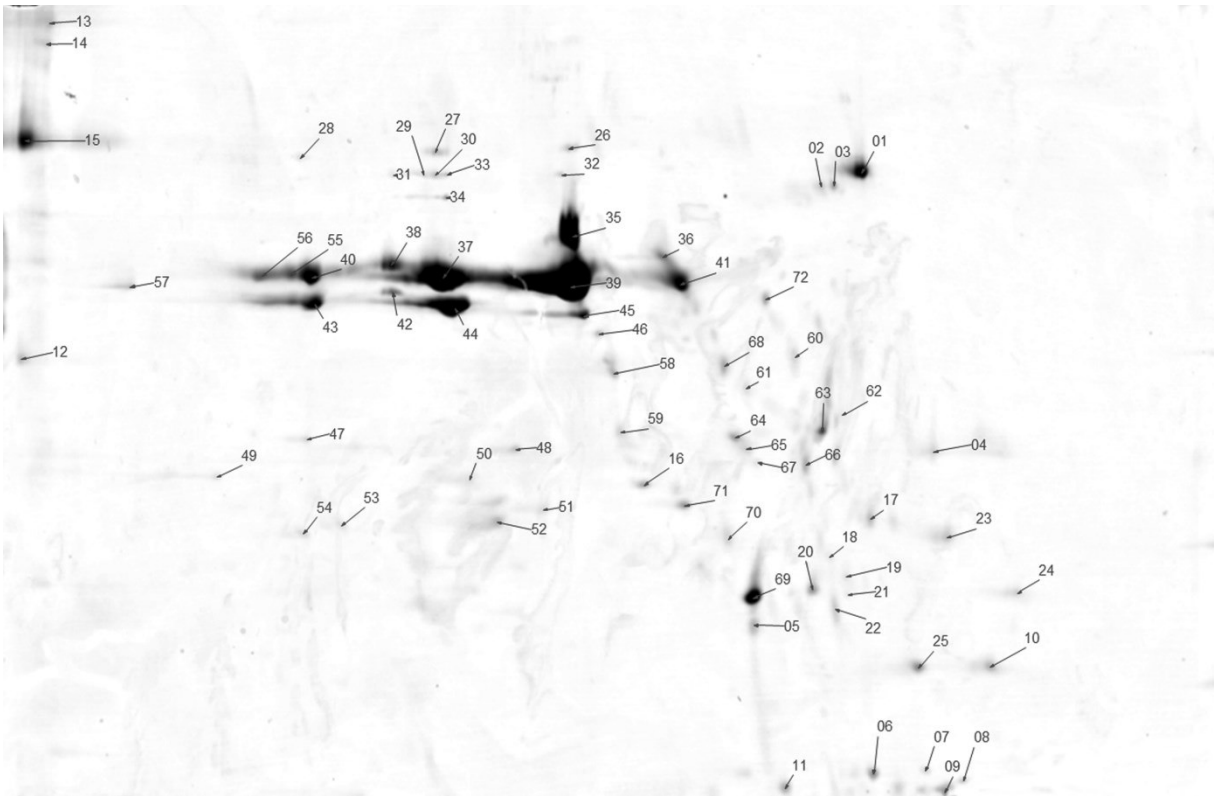


Figure S-3 Spot labels of 2D gels

Table S-1: List of proteins identified in *C. albicans* SC5314 culture supernatants identified in at least 2 replicates with more than 2 peptides in one of them, ordered according to their predicted biological process. Secretomes of yeast form and hyphal form were analyzed separately.

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
Cell wall associated proteins								
orf19.1597	ABG1	Vacuolar membrane protein			√	(6,6,7,7)	Y	N
orf19.5007	ACT1	Actin			√	(8,10,13,11)	N	N
orf19.3997	ADH1	Alcohol dehydrogenase			√	(10,16,13,14)	N	N
orf19.2762	AHP1	Alkyl hydroperoxide reductase			√	(4,4,4,5)	N	N
orf19.6214	ATC1	Cell wall acid trehalase		(4,3,3,3)		(3,4,6,3)	N	N
orf19.6854	ATP1	ATP synthase alpha subunit				(11,9,13,12)	N	N
orf19.4565	BGL2	Cell wall 1,3-beta-glucosyltransferase	√	(14,14,14,15)		(11,11,14,10)	Y	N
orf19.3014	BMH1	Sole 14-3-3 protein in C. albicans			√	(5,9,10,8)	N	N
orf19.6229	CAT1	Catalase				(3,2,3,3)	N	N
orf19.3575	CDC19	Pyruvate kinase at yeast cell surface			√	(13,16,12,14)	N	N
orf19.1055	CDC3	Septin				(2,2,3,2)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.4152	CEF3	Translation elongation factor 3				(13,10,19,10)	N	N
orf19.7517	CHT1	Chitinase		(4,5,5,5)			Y	Y
orf19.3895	CHT2	GPI-linked chitinase		(10,10,10,11)		(10,11,12,11)	Y	Y
orf19.7586	CHT3	Major chitinase		(7,7,7,8)	√	(6,6,6,6)	Y	N
orf19.2706	CRH11	GPI-anchored cell wall transglycosylase		(11,8,8,11)		(10,12,12,12)	Y	Y
orf19.4477	CSH1	Aldo-keto reductase				(3,2,2,3)	N	N
orf19.3010.1	ECM33	GPI-anchored cell wall protein		(8,8,8,8)		(6,9,9,7)	Y	Y
orf19.4255	ECM331	GPI-anchored protein				(2,2,2,2)	Y	N
orf19.5788	EFT2	Elongation factor 2			√	(26,34,34,32)	N	N
orf19.3066	ENG1	Endo-1,3-beta-glucanase		(26,24,24,27)		(7,13,13,11)	Y	N
orf19.395	ENO1	Enolase		(6,3,6,6)	√	(19,20,21,20)	N	N
orf19.2952	EXG2	GPI-anchored cell wall protein				(3,3,4)	Y	Y
orf19.4618	FBA1	Fructose-bisphosphate aldolase			√	(14,17,17,17)	N	N
orf19.251	GLX3	Glutathione-independent glyoxalase		(3,2,2,2)	√	(11,12,10,11)	N	N
orf19.7021	GPH1	Putative glycogen phosphorylase				(4,3,3,5)	N	N
orf19.903	GPM1	Phosphoglycerate mutase			√	(8,11,10,10)	N	N
orf19.2803	HEM13	Coproporphyrinogen III oxidase			√	(3,7,6,5)	N	N
orf19.4980	HSP70	Putative hsp70 chaperone			√	(28,30,28,27)	N	N
orf19.6515	HSP90	Essential chaperone		(2,3,3,4)	√	(27,33,34,31)	N	N
orf19.4975	HYR1	GPI-anchored hyphal cell wall protein				(3,7,6,6)	Y	Y
orf19.5399	IFF11	Secreted protein		(2,3,3)			Y	N
orf19.7585	INO1	Inositol-1-phosphate synthase			√	(8,13,14,13)	N	N
orf19.3590	IPP1	Putative inorganic pyrophosphatase			√	(10,11,12,12)	N	N
orf19.4755	KEX2	Subtilisin-like protease		(5,5,8,7)	√	(9,12,16,14)	Y	N
orf19.290	KRE5	UDP-glucose:glycoprotein glucosyltransferase				(5,6,10,5)	N	N
orf19.5645	MET15	O-acetylhomoserine O-acetylserine sulphydrylase			√	(11,14,12,12)	N	N
orf19.2551	MET6	Cobalamin-independent methionine synthase		(4,6,6,5)	√	(37,39,40,39)	N	N
orf19.1665	MNT1	Alpha-1,2-mannosyl transferase		(5,4,6,5)		(8,12,17,11)	N	N
orf19.1663	MNT2	Alpha-1,2-mannosyl transferase				(4,5,9,4)	N	N
orf19.1779	MP65	Cell surface mannoprotein	√	(16,17,18,18)	√	(15,17,16,16)	Y	N
orf19.1490	MSB2	Mucin family adhesin-like protein		(9,8,8,9)		(7,8,9,7)	Y	N
orf19.2677	orf19.2677	Putative GPI transamidase component				(6,6,9,8)	Y	N
orf19.6741	orf19.6741	Putative plasma membrane protein		(3,3,4,5)			N	N
orf19.2877	PDC11	Pyruvate decarboxylase			√	(10,17,16,16)	N	N
orf19.4035	PGA4	GPI-anchored cell surface protein		(13,12,10,12)		(8,9,10,8)	Y	Y
orf19.2451	PGA45	Putative GPI-anchored cell wall protein		(4,4,4,4)		(4,5,4,4)	Y	Y
orf19.1911	PGA52	GPI-anchored cell surface protein of unknown function		(2,2,2,2)			Y	Y
orf19.3651	PGK1	Phosphoglycerate kinase			√	(21,27,25,25)	N	N
orf19.3829	PHR1	Cell surface glycosidase				(18,21,22,21)	Y	Y
orf19.6081	PHR2	Glycosidase		(7,8,7,8)			Y	Y
orf19.220	PIR1	1,3-beta-glucan-linked cell wall protein		(2,2,2,2)			N	N
orf19.1390	PMI1	Phosphomannose isomerase			√		N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.3765	RAX2	Plasma membrane protein involved in establishment of bud sites and linear direction of hyphal growth				(6,10,12,8)	Y	N
orf19.1327	RBT1	Cell wall protein with similarity to Hwp1			√	(7,7,12,9)	Y	Y
orf19.5305	RHD3	GPI-anchored yeast-associated cell wall protein		(9,9,9,9)		(4,7,6,5)	Y	N
orf19.2843	RHO1	Small GTPase of Rho family		(5,5,5,4)		(3,4,8,3)	N	N
orf19.4660	RPS6A	Ribosomal protein 6A				(3,3,5,5)	N	N
orf19.657	SAM2	S-adenosylmethionine synthetase			√	(9,13,17,14)	N	N
orf19.3893	SCW11	Cell wall protein	√	(13,13,14,13)	√	(10,11,10,11)	Y	N
orf19.5032	SIM1	Adhesin-like protein	√	(14,15,15,15)		(9,8,9,8)	Y	N
orf19.2770.1	SOD1	Superoxide dismutase [Cu-Zn]			√	(3,5,4,3)	N	N
orf19.2060	SOD5	Cu and Zn-containing superoxide dismutase				(5,6,6,6)	Y	Y
orf19.6190	SRB1	Essential GDP-mannose pyrophosphorylase				(5,7,8,4)	N	N
orf19.8667	SSA2	HSP70 family chaperonin	√		√	(23,26,27,25)	N	N
orf19.6367	SSB1	Hypothetical protein CaO19.6367			√	(16,17,15,18)	N	N
orf19.1896	SSC1	Heat shock protein				(6,6,10,9)	N	N
orf19.4772	SSU81	Predicted adaptor protein involved in activation of MAP kinase-dependent signaling pathways		(3,3,3,3)			N	N
orf19.3642	SUN41	Cell wall glycosidase	√	(12,8,9,12)		(9,11,10,11)	Y	N
orf19.3414	SUR7	Protein required for normal cell wall, plasma membrane, cytoskeletal organization, endocytosis		(3,3,3,3)			N	N
orf19.6814	TDH3	NAD-linked glyceraldehyde-3-phosphate dehydrogenase		(3,2,4,4)	√	(15,18,19,17)	N	N
orf19.1435	TEF1	Probable translation elongation factor EF-1 alpha			√		N	N
orf19.5112	TKL1	Transketolase I			√	(10,14,14,16)	N	N
orf19.1690	TOS1	Protein similar to alpha agglutinin anchor subunit		(12,12,10,12)	√	(14,14,14,14)	Y	N
orf19.6745	TPI1	Triose-phosphate isomerase		(3,3,3,2)	√	(8,10,8,9)	N	N
orf19.7417	TSA1	TSA/alkyl hydroperoxide peroxidase C family protein			√	(7,7,8,8)	N	N
orf19.1738	UGP1	UTP-glucose-1-phosphaturidyl transferase				(5,4,5,6)	N	N
orf19.1671	UTR2	Putative GPI anchored cell wall glycosidase		(5,4,4,3)		(7,9,8,8)	Y	Y
orf19.2990	XOG1	Exo-1,3-beta-glucanase	√	(15,14,13,14)	√	(12,12,14,13)	Y	N
orf19.7676	XYL2	D-xylulose reductase			√	(3,8,4,5)	N	N
orf19.6481	YPS7	Putative aspartic-type endopeptidase with limited ability to degrade alpha pheromone		(6,5,6,5)		(4,7,6,3)	Y	N
orf19.3618	YWP1	Secreted yeast wall protein	√	(4,5,5,6)		(3,3,2,3)	Y	Y
orf19.7218	RBE1	Pry family cell wall protein		(6,6,5,6)			Y	N
Secreted proteases, lipases and adhesins								
orf19.1339	CPY1	Carboxypeptidase Y			√	(13,15,15,15)	Y	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.1442	PLB4.5	Phospholipase B	√	(24,24,23,24)		(10,17,17,13)	Y	Y
orf19.1816	ALS3	Cell wall adhesin				(10,13,14,12)	Y	Y
orf19.3591	APE3	Putative vacuolar aminopeptidase Y,			√	(19,21,23,20)	Y	N
orf19.5197	APE2	Neutral arginine, alanine, leucine specific metallo-aminopeptidase			√	(13,14,14,14)	N	N
orf19.5542	SAP6	secretory aspartyl proteinase SAP6p			√	(24,30,23,25)	Y	N
orf19.5585	SAP5	Secreted aspartyl proteinase			√	(19,24,22,21)	Y	N
orf19.5716	SAP4	Secreted aspartyl proteinase			√	(9,17,10,15)	Y	N
orf19.5741	ALS1	Cell-surface adhesin				(5,4,4,4)	Y	Y
orf19.690	PLB2	Putative phospholipase B				(2,7,5,4)	Y	N
orf19.6928	SAP9	Secreted aspartyl protease		(4,3,4,4)		(4,6,7,5)	Y	Y
orf19.709	PUP2	Alpha5 subunit of the 20S proteasome			√		N	N
orf19.756	SAP7	Pepstatin A-insensitive secreted aspartyl protease		(14,13,13,13)		(9,10,11,8)	Y	N
orf19.3839	SAP10	Secreted aspartyl protease		(5,3,4,5)			Y	Y
orf19.3708	SAP2	Major secreted aspartyl proteinase		(4,4,3,4)			Y	N
orf19.6001	SAP3	Secreted aspartyl proteinase		(9,8,7,9)			Y	N
orf19.1097	ALS2	ALS family protein		(9,8,8,10)			Y	N
orf19.4555	ALS4	GPI-anchored adhesin		(3,3,2,2)			Y	Y
orf19.2133	LIP4	Secreted lipase	√	(17,15,14,16)			Y	N
Carbohydrate metabolic process								
orf19.5113	ADH2	Alcohol dehydrogenase				(5,7,7,7)	N	N
orf19.5013	AGM1	Phosphoacetylglucosamine mutase				(2,3,3,3)	N	N
orf19.2334	BIG1	Endoplasmic reticulum (ER) protein		(2,2,2,2)			Y	N
orf19.4393	CIT1	Citrate synthase			√	(9,10,18,13)	N	N
orf19.3670	GAL1	Galactokinase				(3,7,5,5)	N	N
orf19.3672	GAL10	UDP-glucose 4-epimerase				(3,6,7,3)	N	N
orf19.4899	GCA1	Extracellular/plasma membrane-associated glucoamylase				(4,13,9)	Y	N
orf19.999	GCA2	Predicted extracellular glucoamylase		(5,2,4,5)		(5,13,10,9)	Y	N
orf19.7394	GDA1	Golgi membrane GDPase		(3,3,3,3)		(4,6,10,5)	N	N
orf19.1618	GFA1	Glucosamine-6-phosphate synthase				(3,2,5,4)	N	N
orf19.734	GLK1	Putative glucokinase			√	(7,12,9,10)	N	N
orf19.4317	GRE3	Putative D-xylose reductase			√	(4,6,4,5)	N	N
orf19.6673	HEX1	Beta-N-acetylhexosaminidase/chitobiase		(7,6,5,8)	√	(15,16,17,15)	Y	N
orf19.6387	HSP104	Heat-shock protein				(8,6,9,10)	N	N
orf19.822	HSP21	Small heat shock protein			√	(5,5,5,5)	N	N
orf19.2154	HXK1	N-acetylglucosamine (GlcNAc) kinase				(3,5,4,3)	N	N
orf19.542	HXK2	Likely hexokinase II			√		N	N
orf19.6844	ICL1	Isocitrate lyase				(5,3,3,3)	N	N
orf19.4475	KTR4	Mannosyltransferase				(2,3,4,2)	N	N
orf19.7080	LEU2	Isopropyl malate dehydrogenase			√	(6,10,10,9)	N	N
orf19.7668	MAL2	Maltase			√	(15,18,21,18)	N	N
orf19.7481	MDH1	Mitochondrial malate dehydrogenase			√	(7,4,6,7)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.4602	MDH1-1	Predicted malate dehydrogenase precursor				(9,9,14,11)	N	N
orf19.4833	MLS1	Malate synthase				(4,2,2,5)	N	N
orf19.2347	MNN2	Alpha-1,2-mannosyltransferase		(2,4,3,3)	√	(10,16,19,14)	N	N
orf19.6692	MNN26	Putative alpha-1,2-mannosyltransferase				(8,10,19,9)	N	N
orf19.1036	MNS1	Alpha-1,2-mannosidase	√				N	N
orf19.2156	NAG1	Glucosamine-6-phosphate deaminase				(6,6,6,5)	N	N
orf19.1946	orf19.1946	Hypothetical protein CaO19.1946			√	(4,5,6,6)	N	N
orf19.3286	orf19.3286	Ortholog(s) have alpha-glucosidase activity				(5,4,6,3)	Y	N
orf19.3982	orf19.3982	Maltase			√	(10,15,17,13)	N	N
orf19.930	PET9	Mitochondrial ADP/ATP carrier protein involved in ATP biosynthesis				(7,6,9,7)	N	N
orf19.3888	PGI1	Glucose-6-phosphate isomerase			√	(12,16,16,15)	N	N
orf19.2841	PGM2	Ortholog of S. cerevisiae Pgm2			√	(9,13,14,12)	N	N
orf19.2937	PMM1	Phosphomannomutase			√	(7,9,12,9)	N	N
orf19.789	PYC2	Putative pyruvate carboxylase				(14,15,18,14)	N	N
orf19.6029	ROT1	Similar to S. cerevisiae Rot1p		(2,2,2,2)			Y	N
orf19.974	ROT2	Alpha-glucosidase II, catalytic subunit			√	(10,12,14,12)	Y	N
orf19.2941	SCW4	Putative cell wall protein		(2,2,2,2)			N	N
orf19.6640	TPS1	Trehalose-6-phosphate synthase				(2,3,3,3)	N	N
orf19.2298	WBP1	Putative oligosaccharyltransferase subunit				(3,3,2)	Y	N
Translation								
orf19.3426	ANB1	Translation initiation factor eIF-5A				(6,6,6,6)	N	N
orf19.7382	CAM1	Putative translation elongation factor eEF1 gamma				(6,4,10,5)	N	N
orf19.2560	CDC60	Cytosolic leucyl tRNA synthetase				(7,9,5,6)	N	N
orf19.6702	DED81	Putative tRNA-Asn synthetase				(2,3,3,2)	N	N
orf19.3838	EFB1	Translation elongation factor EF-1 beta				(3,3,4,3)	N	N
orf19.437	GRS1	Putative tRNA-Gly synthetase				(9,10,15,9)	N	N
orf19.2138	ILS1	Putative isoleucyl-tRNA synthetase				(4,7,9,6)	N	N
orf19.6749	KRS1	Putative tRNA-Lys synthetase				(2,2,3,3)	N	N
orf19.6220.3	MMD1	Mitochondrial protein				(3,3,4,2)	N	N
orf19.2478.1	orf19.2478.1	60S ribosomal protein L7				(5,4,7,3)	N	N
orf19.3341	orf19.3341	Putative tRNA-Arg synthetase				(2,3,5,3)	N	N
orf19.3354	orf19.3354	Ortholog(s) have structural constituent of ribosome activity				(8,8,13,11)	N	N
orf19.3572.3	orf19.3572.3	Ribosomal 60S subunit protein L31B				(3,2,3,3)	N	N
orf19.4149.1	orf19.4149.1	Protein component of the small (40S) ribosomal subunit				(3,3,4,4)	N	N
orf19.6220.4	orf19.6220.4	Ribosomal 60S subunit protein				(3,2,3,2)	N	N
orf19.6415.1	orf19.6415.1	Ortholog(s) have structural constituent of ribosome activity				(2,3,2,3)	N	N
orf19.6701	orf19.6701	Protein with similarity to amino acid-tRNA ligase				(3,3,6,4)	N	N
orf19.2935	RPL10	Ribosomal protein L10				(4,4,4,4)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.3465	RPL10A	L10A ribosomal protein			√	(6,5,7,5)	N	N
orf19.2232	RPL11	Ribosomal protein				(3,2,3,3)	N	N
orf19.1635	RPL12	Likely cytosolic ribosomal protein L12			√	(5,4,7,6)	N	N
orf19.2994	RPL13	Putative ribosomal subunit				(4,5,7,5)	N	N
orf19.4931.1	RPL14	Ribosomal protein L14				(3,3,4,3)	N	N
orf19.493	RPL15A	Putative ribosomal protein				(4,3,5,5)	N	N
orf19.5982	RPL18	Predicted ribosomal protein				(4,5,6,4)	N	N
orf19.5904	RPL19A	Ribosomal protein L19				(3,3,3,3)	N	N
orf19.2309.2	RPL2	Putative 60S ribosomal protein L2				(4,4,7,4)	N	N
orf19.4632	RPL20B	Ribosomal protein L20				(2,2,3,3)	N	N
orf19.687.1	RPL25	Putative rRNA-binding ribosomal protein component				(2,2,2)	N	N
orf19.5225.2	RPL27A	Ribosomal protein L27				(3,3,6,4)	N	N
orf19.2864.1	RPL28	Putative ribosomal protein				(3,2,5,4)	N	N
orf19.1601	RPL3	Ribosomal protein, large subunit				(6,5,10,7)	N	N
orf19.3415.1	RPL32	Component of the large (60S) ribosomal subunit				(2,2,5,4)	N	N
orf19.827.1	RPL39	Ribosomal protein L39				(2,3,3,3)	N	N
orf19.7217	RPL4B	Ribosomal protein 4B				(4,6,8,5)	N	N
orf19.6541	RPL5	Ribosomal protein				(4,3,4,3)	N	N
orf19.236	RPL9B	Ribosomal protein L9			√	(4,2,5,4)	N	N
orf19.7015	RPP0	Putative ribosomal protein			√	(6,6,6,4)	N	N
orf19.6403.1	RPP2A	Acidic ribosomal protein				(3,3,3,2)	Y	N
orf19.3002	RPS1	Putative ribosomal protein 10 of the 40S subunit				(6,6,12,6)	N	N
orf19.6785	RPS12	Acidic ribosomal protein S12				(4,5,5,3)	N	N
orf19.4193.1	RPS13	Putative ribosomal protein of the small subunit				(4,3,4,3)	N	N
orf19.6265.1	RPS14B	Putative ribosomal protein				(3,2,3,3)	N	N
orf19.5927	RPS15	Putative ribosomal protein				(2,2,2,2)	N	N
orf19.2994.1	RPS16A	Putative 40S ribosomal subunit				(3,3,5,3)	N	N
orf19.2329.1	RPS17B	Ribosomal protein 17B				(4,3,5,4)	N	N
orf19.7018	RPS18	Predicted ribosomal protein				(7,5,9,7)	N	N
orf19.5996.1	RPS19A	Putative ribosomal protein S19			√	(3,3,4,4)	N	N
orf19.6375	RPS20	Putative ribosomal protein				(4,4,5,5)	N	N
orf19.3334	RPS21	Protein component of the small (40S) subunit				(4,4,5,4)	N	N
orf19.3325.3	RPS21B	Ribosomal protein S21				(3,4,3,4)	N	N
orf19.6265	RPS22A	Predicted ribosomal protein				(3,3,3,3)	N	N
orf19.6253	RPS23A	Putative ribosomal protein				(3,3,2,2)	N	N
orf19.5466	RPS24	Predicted ribosomal protein				(3,3,5,3)	N	N
orf19.6286.2	RPS27	Putative ribosomal protein				(3,3,2,3)	N	N
orf19.7048.1	RPS28B	Putative ribosomal protein S28B				(3,3,3,3)	N	N
orf19.6312	RPS3	Ribosomal protein S3			√	(8,7,13,9)	N	N
orf19.4336	RPS5	Ribosomal protein S5				(6,6,7,5)	N	N
orf19.1700	RPS7A	Ribosomal protein S7				(7,8,8,8)	N	N
orf19.6873	RPS8A	Small 40S ribosomal subunit protein				(4,4,6,5)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.838.1	RPS9B	Predicted ribosomal protein				(5,4,6,6)	N	N
orf19.269	SES1	Seryl-tRNA synthetase				(3,3,3,2)	N	N
orf19.1280	SUI1	Putative translation initiation factor				(2,2,2,2)	N	N
orf19.382	TEF2	Translation elongation factor 1-alpha			√	(15,16,18,17)	N	N
orf19.3324	TIF	Translation initiation factor			√	(12,18,18,16)	N	N
orf19.3087	UBI3	Fusion of ubiquitin with the S34 protein of the small ribosomal subuni		(5,4,3,5)		(7,9,9,8)	N	N
orf19.6975	YST1	Ribosome-associated protein			√	(6,5,6,5)	N	N
Response to stimuli								
orf19.1891	APR1	Vacuolar aspartic proteinase				(10,12,11,11)	Y	N
orf19.3171	ACH1	Acetyl-coA hydrolase			√	(5,5,8,8)	N	N
orf19.7484	ADE1	Phosphoribosylaminoimadazole succinocarboxamide synthetase				(3,3,3,3)	N	N
orf19.3870	ADE13	Adenylosuccinate lyase				(4,5,7,5)	N	N
orf19.5591	ADO1	Adenosine kinase			√	(7,9,9,9)	N	N
orf19.7602	AHA1	Putative Hsp90p co-chaperone				(4,3,4,4)	N	N
orf19.5964	ARF2	Putative ADP-ribosylation factor		(3,3,4,4)	√	(5,8,8,8)	N	N
orf19.6906	ASC1	40S ribosomal subunit similar to G-beta subunits		(3,3,7,5)	√	(9,10,8,12)	N	N
orf19.5641	CAR2	Ornithine aminotransferase				(3,3,4,3)	N	N
orf19.2340	CDC48	Putative microsomal ATPase	√			(8,4,5,9)	N	N
orf19.2157	DAC1	N-acetylglucosamine-6-phosphate (GlcNAcP) deacetylase				(3,5,4,3)	N	N
orf19.4688	DAG7	Secretory protein		(6,5,5,6)		(4,4,4,4)	Y	N
orf19.2613	ECM4	Cytoplasmic glutathione S-transferase				(4,5,3,4)	N	N
orf19.7312	ERG13	3-hydroxy-3-methylglutaryl coenzyme A synthase				(3,3,5,3)	N	N
orf19.979	FAS1	Beta subunit of fatty-acid synthase				(21,21,26,15)	N	N
orf19.4215	FET34	Multicopper ferroxidase				(3,3,2,2)	Y	N
orf19.1153	GAD1	Putative glutamate decarboxylase				(9,5,3,8)	N	N
orf19.5024	GND1	6-phosphogluconate dehydrogenase			√	(19,25,28,23)	N	N
orf19.86	GPX1	Potential glutathione peroxidase/redox transducer			√		N	N
orf19.2727	GRX3	Putative glutaredoxin				(2,3,2,2)	N	N
orf19.717	HSP60	Heat shock protein				(10,9,11,12)	N	N
orf19.2013	KAR2	Similar to Hsp70 family chaperones		(20,17,16,20)	√	(34,36,40,35)	N	N
orf19.5830	LHS1	Protein similar to S. cerevisiae Hsp70p		(5,5,6,7)		(13,16,23,17)	N	N
orf19.3507	MCR1	NADH-cytochrome-b5 reductase				(8,6,5,5)	N	N
orf19.2435	MSI3	Essential HSP70 family protein				(11,13,18,15)	N	N
orf19.2028	MXR1	Putative methionine sulfoxide reductase				(3,3,3,2)	N	N
orf19.2304	orf19.2304	Protein similar to S. cerevisiae Gvp36p	√				N	N
orf19.3438	orf19.3438	Ortholog(s) have chaperone binding activity				(2,2,4,3)	Y	N
orf19.4150	orf19.4150	Putative glutaredoxin				(2,2,3,2)	Y	N
orf19.5322	orf19.5322	Phosphatidylinositol-3-phosphate binding activity		(5,3,4,4)			Y	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.7578	orf19.7578	Ortholog(s) have oligosaccharide binding activity		(3,2,3,3)		(4,5,5,4)	Y	N
orf19.3727	PHO112	Putative constitutive acid phosphatase		(6,11,7,9)			N	N
orf19.5383	PMA1	Plasma membrane H(+)-ATPase				(8,8,9,8)	N	N
orf19.12086	POL30	Similar to proliferating cell nuclear antigen (PCNA)			√		N	N
orf19.5180	PRX1	Thioredoxin peroxidase			√	(9,12,11,11)	N	N
orf19.3702	RBP1	Peptidyl-prolyl cis-trans isomerase			√	(2,3,3,3)	N	N
orf19.6202	RBT4	Pry family protein		(5,5,4,5)	√	(7,10,9,8)	Y	N
orf19.5968	RDI1	Putative rho GDP dissociation inhibitor			√	(8,10,11,9)	N	N
orf19.6085	RPL16	Ribosomal protein L16			√	(3,2,6,2)	N	N
orf19.4490	RPL17B	Ribosomal protein L17				(3,2,3,2)	N	N
orf19.3003.1	RPL6	Ortholog of S. cerevisiae ribosomal subunit, Rpl6B				(4,4,5,4)	N	N
orf19.5823	SGT2	Putative small tetratricopeptide repeat (TPR)-containing protein				(4,5,5,4)	N	N
orf19.670	SMT3	SUMO, small ubiquitin-like protein				(3,4,3,3)	N	N
orf19.3340	SOD2	Mitochondrial Mn-containing superoxide dismutase				(3,2,2,2)	N	N
orf19.1453	SPT5	Transcription elongation factor			√		N	N
orf19.5647	SUB2	Putative TREX complex component with a predicted role in nuclear mRNA export				(3,3,3,3)	N	N
orf19.6068	SVF1	Putative survival factor				(2,2,3,3)	N	N
orf19.3268	TMA19	Hypothetical protein CaO19.3268			√	(4,5,4,5)	N	N
orf19.4290	TRR1	Thioredoxin reductase			√	(3,3,3,3)	N	N
orf19.6059	TTR1	Putative glutaredoxin				(3,3,3,3)	N	N
orf19.7308	TUB1	Alpha-tubulin				(5,6,7,5)	N	N
orf19.6034	TUB2	Beta-tubulin				(5,4,5,4)	N	N
orf19.6109	TUP1	Transcriptional corepressor				(3,3,3,4)	N	N
orf19.4311	YNK1	Nucleoside diphosphate kinase (NDP kinase)			√	(6,7,7,7)	N	N
orf19.4754	ZWF1	Glucose-6-phosphate dehydrogenase			√	(10,10,9,12)	N	N
orf19.7196	PRB12	Hypothetical protein CaO19.7196			√		Y	N
vesicle-mediated transport								
orf19.953.1	COF1	Putative cofilin			√	(7,7,7,6)	N	N
orf19.6293	EMP24	COPII-coated vesicle component				(3,2,3,2)	Y	N
orf19.731	EMP46	Protein similar to S. cerevisiae Emp46		(3,3,4,4)	√	(11,10,13,11)	Y	N
orf19.7409	ERV25	Component of COPII-coated vesicles		(4,3,3,4)		(2,3,2,3)	Y	N
orf19.3149	LSP1	Eisosome component with a predicted role in endocytosis				(4,4,5,4)	N	N
orf19.2416.1	MLC1	Microtubule-dependent localized protein				(4,5,5,4)	N	N
orf19.2168.3	orf19.2168.3	Ortholog(s) have role in cellular protein localization		(2,2,2,2)			N	N
orf19.3767	PEP1	Type I transmembrane sorting receptor for multiple vacuolar hydrolases		(5,6,5,6)		(7,8,15,5)	N	N
orf19.4236	RET2	Delta subunit of the coatomer complex (COPI)				(3,2,3)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.3462	SAR1	Functional homolog of S. cerevisiae Sar1				(2,4,6,3)	N	N
orf19.1680	TFP1	Subunit of vacuolar H+-ATPase				(4,3,2,3)	N	N
orf19.7611	TRX1	Thioredoxin		(4,4,4,4)		(4,5,4,4)	N	N
orf19.2974	YKT6	Putative protein of the vacuolar SNARE complex				(5,8,8,5)	N	N
orf19.3052	YPT1	Functional homolog of S. cerevisiae Ypt1p				(3,3,2,3)	N	N
orf19.2622	YPT31	Protein required for resistance to toxic ergosterol analog		(3,3,2,3)		(5,7,5,5)	N	N
orf19.7261	GDI1	Hypothetical protein CaO19.7261			√	(3,6,9,5)	N	N
Protein folding								
orf19.6472	CYP1	Peptidyl-prolyl cis-trans isomerase		(3,2,2,3)		(6,9,8,8)	N	N
orf19.7421	CYP5	Putative peptidyl-prolyl cis-trans isomerase		(8,8,7,8)	√	(12,13,13,13)	Y	N
orf19.4871	ERO1	Ortholog of S. cerevisiae Ero1		(3,3,3,3)		(4,4,5,2)	Y	N
orf19.3396	HCH1	Ortholog of S. cerevisiae Hch1				(3,4,3,2)	N	N
orf19.4952.1	orf19.4952.1	Ortholog(s) have FK506 binding, Hsp70 protein binding	√	(3,3,3,3)	√	(4,5,5,4)	Y	N
orf19.7215.3	orf19.7215.3	Chaperone binding, unfolded protein binding activity				(4,5,5,4)	N	N
orf19.5130	PDI1	Putative protein disulfide-isomerase		(13,11,13,13)	√	(28,29,30,29)	Y	N
orf19.3192	STI1	Protein that interacts with Cdc37 and Crk1 in two-hybrid				(9,8,8,9)	N	N
Regulation of biological process								
orf19.2699	ABP1	Ortholog of S. cerevisiae Abp1				(5,6,7,7)	N	N
orf19.6385	ACO1	Aconitase			√	(7,10,15,11)	N	N
orf19.1282	CKS1	Ortholog(s) have histone binding, protein complex binding, protein homodimerization activity				(2,2,3,3)	N	N
orf19.7654	CPR6	Putative peptidyl-prolyl cis-trans isomerase				(4,4,4,4)	N	N
orf19.5949	FAS2	Alpha subunit of fatty-acid synthase				(12,10,18,9)	N	N
orf19.5493	GSP1	Small RAN G-protein			√	(3,5,5,5)	N	N
orf19.6127	LPD1	Putative dihydrolipoamide dehydrogenase			√	(7,3,6,5)	N	N
orf19.3294	MBF1	Putative transcriptional coactivator				(3,4,4,2)	N	N
orf19.13277	MBP1	Putative component of the MBF transcription complex involved in G1/S cell-cycle progression			√		N	N
orf19.1448.1	orf19.1448.1	Ortholog(s) have plus-end-directed microtubule motor activity				(0,2,2,2)	N	N
orf19.2489	orf19.2489	Putative karyopherin beta				(2,4,6,3)	N	N
orf19.2757	orf19.2757	Has domain(s) with predicted role in cell redox homeostasis				(6,8,11,8)	Y	N
orf19.2769	orf19.2769	Putative protease B inhibitor		(3,3,3,3)		(3,3,3,4)	N	N
orf19.3037	orf19.3037	Putative poly(A)-binding protein				(8,9,5,8)	N	N
orf19.5201.1	orf19.5201.1	Has domain(s) with predicted enzyme inhibitor activity				(3,2,3,2)	N	N
orf19.5076	PFY1	Profilin				(4,5,5,4)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.5956	PIN3	Putative SH3-domain-containing protein				(3,3,3,2)	Y	N
orf19.1042	POR1	Mitochondrial outer membrane porin				(3,4,7,6)	N	N
orf19.2242	PRB1	Endoprotease B				(9,11,12,11)	N	N
orf19.2241	PST1	Putative 1,4-benzoquinone reductase				(2,2,2)	N	N
orf19.5285	PST3	Putative flavodoxin			√	(4,6,6,4)	N	N
orf19.1649	RNA1	Putative GTPase-activating protein				(3,2,3,2)	N	N
orf19.5544	SAC6	Fimbrin				(4,3,3,3)	N	N
orf19.1974	TFS1	Putative carboxypeptidase y inhibitor		(3,2,3)	√	(4,3,3,3)	N	N
orf19.754	YBN5	P-loop ATPase with similarity to human OLA1 and bacterial YchF				(2,5,5,3)	N	N
orf19.7477	YRB1	Functional homolog of S. cerevisiae Yrb1p			√		N	N
orf19.6472	CYP1	Peptidyl-prolyl cis-trans isomerase		(3,2,2,3)		(6,9,8,8)	N	N
orf19.5130	PDI1	Putative protein disulfide-isomerase		(13,11,13,13)	√	(28,29,30,29)	Y	N
lipid metabolic process								
orf19.1591	ERG10	Acetyl-CoA acetyltransferase				(5,10,8,8)	N	N
orf19.4491	ERG20	Putative farnesyl pyrophosphate synthetase				(3,5,4,3)	N	N
orf19.2775	IDI1	Ortholog(s) have isopentenyl-diphosphate delta-isomerase activity				(4,8,6,4)	N	N
orf19.6105	MVD	Mevalonate diphosphate decarboxylase				(5,7,5,5)	N	N
orf19.100	orf19.100	Alpha/beta hydrolase and lipase domain protein				(4,4,5,3)	Y	N
orf19.3911	SAH1	S-adenosyl-L-homocysteine hydrolase				(10,12,17,13)	N	N
cellular respiration								
orf19.1471	COX4	Putative cytochrome c oxidase subunit IV				(2,5,3,3)	N	N
orf19.1770	CYC1	Cytochrome c				(4,6,6,5)	N	N
orf19.6724	FUM12	Putative fumarate hydratase				(3,3,7,5)	N	N
orf19.4826	IDH1	Putative mitochondrial NAD-isocitrate dehydrogenase subunit 1				(6,2,7,5)	N	N
orf19.5791	IDH2	Putative mitochondrial NAD-isocitrate dehydrogenase subunit			√	(7,4,6,6)	N	N
transport								
orf19.7043.1	ACB1	Protein similar to a region of acyl-coenzyme-A-binding protein				(2,4,4,2)	N	N
orf19.5653	ATP2	F1 beta subunit of F1F0 ATPase complex			√	(12,10,15,13)	N	N
orf19.5858	EGD2	Nascent polypeptide associated complex protein alpha subunit			√	(2,3,2,2)	N	N
orf19.2322.3	ERP5	Protein involved in ER to Golgi transport				(3,5,3,3)	Y	N
orf19.6327	HET1	Putative sphingolipid transfer protein		(4,3,3,4)		(5,7,6,7)	N	N
orf19.4879.2	NTF2	Putative nuclear envelope protein				(3,3,2,3)	N	N
orf19.6570	NUP	Nucleoside permease		(2,2,3,3)			Y	N
orf19.3219	orf19.3219	Ortholog of S. cerevisiae Sia1				(5,5,5,3)	N	N
orf19.3226	orf19.3226	Ortholog(s) have role in intracellular sterol transport and extracellular region		(4,3,4,4)		(4,4,5,4)	Y	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.590	orf19.590	Putative thiamine biosynthesis enzyme				(3,4,4,3)	N	N
orf19.6403	orf19.6403	Ortholog(s) have adenyl-nucleotide exchange factor activity			√	(8,9,11,10)	Y	N
orf19.7438	UBA1	Ubiquitin-activating enzyme				(4,8,9,6)	N	N
organelle organization								
orf19.1064	ACS2	Acetyl-CoA synthetase			√	(11,14,16,14)	N	N
orf19.88	ILV5	Ketol-acid reductoisomerase			√	(4,5,8,6)	N	N
orf19.3799	orf19.3799	Ortholog(s) have role in endoplasmic reticulum tubular network maintenance, nuclear pore complex assembly and Golgi apparatus, cortical endoplasmic reticulum				(3,4,4,2)	N	N
orf19.7310	orf19.7310	Protein with a role in directing meiotic recombination events to homologous chromatids		(3,2,4,3)			Y	N
orf19.2769	orf19.2769	Putative protease B inhibitor		(3,3,3,3)		(3,3,3,4)	N	N
orf19.3087	UBI3	Fusion of ubiquitin with the S34 protein of the small ribosomal subuni		(5,4,3,5)		(7,9,9,8)	N	N
unknown function								
orf19.5063	COI1	Secreted protein	√	(9,9,9,9)		(9,9,11,10)	Y	N
orf19.1239	orf19.1239	Secreted protein		(5,6,7,5)		(5,0,5,4)	Y	N
orf19.1350	orf19.1350	Protein with a thioredoxin domain				(2,2,3,2)	N	N
orf19.1383	orf19.1383	Protein of unknown function			√		N	N
orf19.1533	orf19.1533	Possible vacuolar protein				(2,2,2,3)	Y	N
orf19.1766	orf19.1766	Secreted protein; fluconazole-induced		(4,4,4,4)			Y	N
orf19.2036	orf19.2036	Predicted dihydrodiol dehydrogenase				(4,4,5,4)	N	N
orf19.2125	orf19.2125	Protein of unknown function				(6,5,5,5)	N	N
orf19.2452	orf19.2452	Protein of unknown function				(3,3,5,3)	Y	N
orf19.2460	orf19.2460	Protein of unknown function		(2,3,3,3)		(4,6,7,4)	Y	N
orf19.2645	orf19.2645				√		N	N
orf19.3004	orf19.3004	Ortholog(s) have fungal-type vacuole localization				(4,4,5,4)	Y	N
orf19.3053	orf19.3053	Protein of unknown function			√	(4,5,4,4)	N	N
orf19.3173	orf19.3173				√	(8,10,11,10)	Y	N
orf19.3475	orf19.3475	Described as a Gag-related protein				(4,4,4,3)	N	N
orf19.3499	orf19.3499	Secreted potein				(4,6,4,4)	Y	N
orf19.3910	orf19.3910	Has domain(s) with predicted RNA binding, ribonuclease T2 activity				(7,11,11,10)	Y	N
orf19.3932	orf19.3932	Predicted RNA binding protein				(3,2,3,3)	N	N
orf19.4376	orf19.4376	Protein of unknown function	√				N	N
orf19.4609	orf19.4609	Putative dienelactone hydrolase				(4,7,4,7)	N	N
orf19.4787	orf19.4787	Ortholog(s) have cytoplasm, nucleus localization	√				N	N
orf19.5925	orf19.5925			(2,3,4,3)		(3,4,4,4)	N	N
orf19.6119	orf19.6119	Protein of unknown function		(8,7,7,7)			N	N
orf19.6200	orf19.6200	Pry family pathogenesis-related protein				(3,5,4,4)	Y	N
orf19.6487	orf19.6487			(3,3,3,3)			Y	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.6553	orf19.6553	Hypothetical protein CaO19.6553			√	(14,14,16,14)	Y	N
orf19.6809	orf19.6809	Putative phosphomutase-like protein				(3,4,6,3)	N	N
orf19.6867	orf19.6867	Protein with a predicted cytochrome b5-like heme/steroid binding domain				(2,3,2,2)	N	N
orf19.7297	orf19.7297	Putative cystathionine gamma-synthase				(4,4,6,5)	N	N
orf19.2619	PHO113	Putative constitutive acid phosphatase		(6,9,8,9)			N	N
orf19.2681	RBT7	Protein with similarity to RNase T2 enzymes		(2,2,2,3)			Y	N
orf19.7350	RCT1	Fluconazole-induced protein			√	(7,8,9,9)	N	N
orf19.3789	RPL24A	Predicted ribosomal protein				(3,2,4,2)	N	N
orf19.6663	RPS25B	Ribosomal protein				(2,2,3,3)	N	N
orf19.5078	OFR1	Protein of unknown function				(3,4,5,4)	N	N
Others								
orf19.3554	AAT1	Aspartate aminotransferase				(4,3,6,5)	N	N
orf19.6287	AAT2	Potential aspartate aminotransferase			√	(8,8,8,8)	N	N
orf19.492	ADE17	5-Aminoimidazole-4-carboxamide ribotide transformylase			√	(10,9,9,8)	N	N
orf19.3391	ADK1	Putative adenylate kinase				(3,3,4,2)	N	N
orf19.5806	ALD5	NAD-aldehyde dehydrogenase			√	(12,10,16,14)	N	N
orf19.7469	ARG1	Argininosuccinate synthase			√	(6,8,8,6)	N	N
orf19.1986	ARO2	Putative chorismate synthase			√		N	N
orf19.1517	ARO3	3-deoxy-D-arabinoheptulosonate-7-phosphate synthase				(4,4,4,5)	N	N
orf19.2098	ARO8	Aromatic transaminase aromatic alcohol biosynthesis				(8,4,8,4)	N	N
orf19.5292	AXL2	Ortholog of S. cerevisiae Axl2				(4,3,3,3)	N	N
orf19.6994	BAT22	Putative branched chain amino acid aminotransferase			√	(4,6,7,4)	N	N
orf19.6402	CYS3	Cystathionine gamma-lyase				(4,6,5,5)	N	N
orf19.3322	DUT1	dUTP pyrophosphatase			√		N	N
orf19.3374	ECE1	Hypha-specific protein				(16,17,21,15)	N	N
orf19.299	ECM14	Ortholog(s) have endoplasmic reticulum, fungal-type vacuole localization			√	(24,25,26,25)	Y	N
orf19.7600	FDH3	Glutathione-dependent formaldehyde dehydrogenase				(4,7,6,5)	N	N
orf19.4212	FET99	Multicopper oxidase family protein		(4,4,5,4)			Y	N
orf19.2192	GDH2	Putative NAD-specific glutamate dehydrogenase				(3,3,3,5)	N	N
orf19.4716	GDH3	NADP-glutamate dehydrogenase			√	(13,16,13,14)	N	N
orf19.6058	GLO1	Putative monomeric glyoxalase I				(3,3,3,3)	N	N
orf19.4309	GRP2	Methylglyoxal reductase			√	(8,8,8,10)	N	N
orf19.1078	HBR2	Putative alanine glyoxylate aminotransferase			√		N	N
orf19.4026	HIS1	ATP phosphoribosyl transferase				(3,2,3,2)	N	N
orf19.1559	HOM2	Aspartate-semialdehyde dehydrogenase				(5,7,7,6)	N	N
orf19.2951	HOM6	hypothetical protein CaO19.2951			√	(4,5,6,5)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.5832	HPT1	Putative hypoxanthine-guanine phosphoribosyltransferase				(3,2,3,2)	N	N
orf19.5211	IDP1	Putative isocitrate dehydrogenase				(6,5,4,5)	N	N
orf19.3733	IDP2	Isocitrate dehydrogenase			√	(9,13,13,13)	N	N
orf19.2396	IFR2	Zinc-binding dehydrogenase			√	(2,3,2,3)	N	N
orf19.6561	LAT1	Putative dihydrolipoamide acetyltransferase component (E2) of pyruvate dehydrogenase complex				(3,2,5,3)	N	N
orf19.7498	LEU1	3-isopropylmalate dehydratase				(2,3,4,3)	N	N
orf19.3358	LSC1	Putative succinate-CoA ligase subunit				(3,3,7,4)	N	N
orf19.1860	LSC2	Putative succinate-CoA ligase beta subunit				(3,3,4,3)	N	N
orf19.1789.1	LYS1	Saccharopine dehydrogenase (biosynthetic)				(6,8,8,9)	N	N
orf19.2525	LYS12	Homoisocitrate dehydrogenase				(3,3,3,2)	N	N
orf19.4506	LYS22	Putative homocitrate synthase				(5,3,3,5)	N	N
orf19.7448	LYS9	Saccharopine dehydrogenase			√	(7,6,7,5)	N	N
orf19.2364	MIS11	Predicted mitochondrial C1-tetrahydrofolate synthase precursor				(6,5,12,9)	N	N
orf19.1366	orf19.1366				√		N	N
orf19.1862	orf19.1862	Possible stress protein				(3,4,3,3)	N	N
orf19.2269	orf19.2269	Putative 3-phosphoserine phosphatase				(3,4,5,3)	N	N
orf19.2516	orf19.2516	Has domain(s) with predicted role in cell redox homeostasis		(4,3,3,3)			Y	N
orf19.3915	orf19.3915	Putative metallodipeptidase				(4,3,6,7)	N	N
orf19.5206	orf19.5206	Putative chaperone protein	√				N	N
orf19.5342	orf19.5342	Ortholog(s) have cytosol, extracellular region etw				(16,18,18,19)	Y	N
orf19.540	orf19.540	Has domain(s) with predicted palmitoyl-(protein) hydrolase activity				(4,3,4,2)	N	N
orf19.5773	orf19.5773	Hypothetical protein CaO19.5773			√	(6,5,5,4)	N	N
orf19.6596	orf19.6596	Putative esterase			√		N	N
orf19.6630	orf19.6630	Role in ER-associated ubiquitin-dependent protein catabolic process				(5,5,6,6)	Y	N
orf19.6732	orf19.6732	Ortholog(s) have cytosol, nucleus localization	√				N	N
orf19.6882	OSM1	Putative flavoprotein subunit of fumarate reductase				(2,6,4,4)	Y	N
orf19.5294	PDB1	Putative pyruvate dehydrogenase				(3,2,3,3)	N	N
orf19.984	PHO8	Putative repressible vacuolar alkaline phosphatase				(3,2,4,3)	Y	N
orf19.317	PNP1	Purine nucleoside phosphorylase			√		N	N
orf19.4135	PRC2	Putative carboxypeptidase		(4,4,2,2)		(11,13,12,12)	Y	N
orf19.2474	PRC3	Putative carboxypeptidase Y precursor				(12,13,12,13)	Y	N
orf19.434	PRD1	Putative proteinase				(5,8,11,6)	N	N
orf19.7178	PRE5	Alpha6 subunit of the 20S proteasome			√		N	N
orf19.4024	RIB5	Putative riboflavin synthase				(3,3,2,2)	N	N
orf19.6002	RPL8B	Predicted ribosomal protein				(6,4,11,7)	N	N
orf19.5484	SER1	Putative 3-phosphoserine aminotransferase			√	(5,4,3,5)	N	N

	Protein	Description	Identified peptides in biological replicates				SP	GPI
			Yeast		Hyphae			
			2D Gel	Gel-free	2D Gel	Gel-free		
orf19.5750	SHM2	Cytoplasmic serine hydroxymethyltransferase				(6,6,9,7)	N	N
orf19.2947	SNZ1	Hypothetical protein CaO19.2947			√	(5,8,5,5)	N	N
orf19.704	SOL3	Putative 6-phosphogluconolactonase				(4,5,6,6)	N	N
orf19.4371	TAL1	Transaldolase			√	(17,20,20,18)	N	N
orf19.4233	THR4	Putative threonine synthase			√	(4,8,9,8)	N	N
orf19.2555	URA5	Putative orotate phosphoribosyltransferase			√	(3,3,2,4)	N	N
orf19.6274	PBR1	hypothetical protein CaO19.6274			√	(3,6,4,4)	N	N

Table S-2 Quantative analysis via TMT labeling

"upregulated" proteins in *C. albicans* yeast cells

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Accession	Gene	Description
22.77	8.54	orf19.3618	YWP1	Secreted yeast wall protein
6.43	2.41	orf19.4688	DAG7	Secretory protein
5.86	2.20	orf19.4555	ALS4	GPI-anchored adhesin
5.81	2.18	orf19.1442	PLB4.5	Phospholipase B
5.63	2.11	orf19.7218	RBE1	Pry family cell wall protein
5.49	2.06	orf19.220	PIR1	1,3-beta-glucan-linked cell wall protein
5.40	2.03	orf19.7517	CHT1	Chitinase

"upregulated" proteins in *C. albicans* hyphal cells

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Ratio H/Y normalized to biomass	Accession	Gene	Description
0.45	0.17	5.95	orf19.3591	APE3	Putative vacuolar aminopeptidase Y
0.45	0.17	5.87	orf19.1327	RBT1	Cell wall protein with similarity to Hwp1
0.46	0.17	5.82	orf19.1816	ALS3	Cell wall adhesin
0.47	0.18	5.62	orf19.7350	RCT1	Fluconazole-induced protein
0.49	0.18	5.43	orf19.1597	ABG1	Vacuolar membrane protein
0.52	0.20	5.12	orf19.6553	orf19.6553	Membrane-localized protein of unknown function
0.54	0.20	4.90	orf19.7421	CYP5	Putative peptidyl-prolyl cis-trans isomerase
0.55	0.20	4.89	orf19.4311	YNK1	Nucleoside diphosphate kinase (NDP kinase)
0.55	0.21	4.87	orf19.2309.2	RPL2	Putative 60S ribosomal protein L2
0.56	0.21	4.78	orf19.5130	PDI1	Putative protein disulfide-isomerase
0.57	0.21	4.69	orf19.1770	CYC1	Cytochrome c
0.57	0.21	4.67	orf19.7571	UBC4	Ortholog(s) have ubiquitin binding activity
0.58	0.22	4.62	orf19.6472	CYP1	Peptidyl-prolyl cis-trans isomerase

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Ratio H/Y normalized to biomass	Accession	Gene	Description
0.58	0.22	4.60	orf19.6085	RPL16A	Ribosomal protein
0.59	0.22	4.50	orf19.6058	GLO1	Putative monomeric glyoxalase I
0.59	0.22	4.49	orf19.5180	PRX1	Thioredoxin peroxidase
0.59	0.22	4.48	orf19.5542	SAP6	Secreted aspartyl protease
0.60	0.23	4.41	orf19.299	ECM14	ER, fungal-type vacuole localization
0.61	0.23	4.39	orf19.5585	SAP5	Secreted aspartyl proteinase
0.61	0.23	4.39	orf19.3268	TMA19	Cell wall protein
0.61	0.23	4.34	orf19.3004	orf19.3004	Fungal-type vacuole localization
0.61	0.23	4.34	orf19.7477	YRB1	Functional homolog of S. cerevisiae Yrb1p
0.63	0.24	4.24	orf19.6274	PBR1	Protein of unknown function
0.63	0.24	4.22	orf19.6403	orf19.6403	Adenyl-nucleotide exchange factor activity
0.65	0.25	4.07	orf19.3475	orf19.3475	A Gag-related protein
0.67	0.25	4.00	orf19.4371	TAL1	Transaldolase
0.67	0.25	3.99	orf19.6814	TDH3	NAD-linked glyceraldehyde-3-phosphate dehydrogenase
0.67	0.25	3.95	orf19.731	EMP46	Protein similar to S. cerevisiae Emp46
0.68	0.25	3.94	orf19.3087	UBI3	Fusion of ubiquitin with the S34 protein of the small ribosomal subunit
0.69	0.26	3.88	orf19.4618	FBA1	Fructose-bisphosphate aldolase
0.69	0.26	3.87	orf19.2757	orf19.2757	Has domain(s) with predicted role in cell redox homeostasis
0.69	0.26	3.84	orf19.1745	orf19.1745	Protein of unknown function
0.71	0.27	3.75	orf19.7196	orf19.7196	Putative vacuolar protease
0.73	0.27	3.67	orf19.382	TEF2	Translation elongation factor 1-alpha
0.73	0.27	3.65	orf19.3426	ANB1	Translation initiation factor eIF-5A
0.74	0.28	3.59	orf19.395	ENO1	Enolase
0.75	0.28	3.57	orf19.3002	RPS1	Putative ribosomal protein 10 of the 40S subunit
0.75	0.28	3.55	orf19.5342	orf19.5342	Cytosol, extracellular region, fungal-type vacuole, nucleus localization
0.75	0.28	3.55	orf19.6673	HEX1	Beta-N-acetylhexosaminidase/chitobiase
0.75	0.28	3.53	orf19.2013	KAR2	Similar to Hsp70 family chaperones
0.76	0.28	3.51	orf19.7668	MAL2	Alpha-glucosidase
0.76	0.29	3.50	orf19.5806	ALD5	NAD-aldehyde dehydrogenase
0.77	0.29	3.45	orf19.903	GPM1	Phosphoglycerate mutase
0.78	0.29	3.44	orf19.3173	orf19.3173	Golgi apparatus, ER, localization
0.78	0.29	3.42	orf19.2994	RPL13	Putative ribosomal subunit
0.78	0.29	3.42	orf19.3888	PGI1	Glucose-6-phosphate isomerase
0.79	0.29	3.39	orf19.3910	orf19.3910	Predicted RNA binding, ribonuclease T2 activity
0.79	0.30	3.38	orf19.1339	CPY1	Carboxypeptidase Y
0.79	0.30	3.37	orf19.1690	TOS1	Protein similar to alpha agglutinin anchor subunit
0.80	0.30	3.35	orf19.2877	PDC11	Pyruvate decarboxylase
0.80	0.30	3.31	orf19.5024	GND1	6-phosphogluconate dehydrogenase
0.81	0.30	3.31	orf19.2551	MET6	cobalamin-independent methionine synthase
0.81	0.30	3.31	orf19.2651	CAM1-1	Putative translation elongation factor
0.81	0.30	3.31	orf19.6253	RPS23A	Putative ribosomal protein

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Ratio H/Y normalized to biomass	Accession	Gene	Description
0.81	0.30	3.30	orf19.5982	RPL18	Predicted ribosomal protein
0.81	0.30	3.29	orf19.4660	RPS6A	Ribosomal protein 6A
0.81	0.30	3.29	orf19.838.1	RPS9B	Predicted ribosomal protein
0.82	0.31	3.27	orf19.5788	EFT2	Elongation Factor 2 (eEF2)
0.82	0.31	3.26	orf19.3982	orf19.3982	Maltase
0.82	0.31	3.26	orf19.3575	CDC19	Pyruvate kinase at yeast cell surface
0.83	0.31	3.22	orf19.3651	PGK1	Phosphoglycerate kinase
0.83	0.31	3.22	orf19.6220.4	orf19.6220.4	Ribosomal 60S subunit protein
0.83	0.31	3.20	orf19.6515	HSP90	Essential chaperone
0.84	0.32	3.16	orf19.979	FAS1	Beta subunit of fatty-acid synthase
0.85	0.32	3.15	orf19.6367	SSB1	HSP70 family heat shock protein
0.85	0.32	3.12	orf19.7600	FDH3	Glutathione-dependent formaldehyde dehydrogenase
0.85	0.32	3.12	orf19.6873	RPS8A	Small 40S ribosomal subunit protein
0.86	0.32	3.11	orf19.3642	SUN41	Cell wall glycosidase
0.86	0.32	3.09	orf19.6854	ATP1	ATP synthase alpha subunit
0.86	0.32	3.09	orf19.3911	SAH1	S-adenosyl-L-homocysteine hydrolase
0.87	0.32	3.08	orf19.4980	HSP70	Putative hsp70 chaperone
0.88	0.33	3.04	orf19.3354	orf19.3354	structural constituent of ribosome activity and 90S preribosome
0.88	0.33	3.04	orf19.3192	STI1	Protein that interacts with Cdc37 and Crk1 in two-hybrid
0.89	0.34	2.98	orf19.1891		Vacuolar aspartic proteinase
0.90	0.34	2.97	orf19.4952.1	orf19.4952.1	FK506 binding, Hsp70 protein binding
0.90	0.34	2.97	orf19.5285	PST3	Putative flavodoxin
0.91	0.34	2.93	orf19.7080	LEU2	Isopropyl malate dehydrogenase
0.91	0.34	2.92	orf19.974	ROT2	Alpha-glucosidase II
0.93	0.35	2.88	orf19.2156	NAG1	Glucosamine-6-phosphate deaminase
0.93	0.35	2.88	orf19.6312	RPS3	Ribosomal protein S3
0.93	0.35	2.88	orf19.4716	GDH3	NADP-glutamate dehydrogenase
0.93	0.35	2.86	orf19.4149.1	orf19.4149.1	Protein component of the small (40S) ribosomal subunit
0.93	0.35	2.85	orf19.5007	ACT1	Actin
0.94	0.35	2.85	orf19.3572.3	orf19.3572.3	Ribosomal 60S subunit protein L31B
0.94	0.35	2.84	orf19.7015	RPP0	Putative ribosomal protein
0.94	0.35	2.84	orf19.2435	MSI3	Essential HSP70 family protein
0.94	0.35	2.82	orf19.3499	orf19.3499	Secreted potein
0.95	0.36	2.81	orf19.5225.2	RPL27A	Ribosomal protein L27
0.95	0.36	2.80	orf19.822	HSP21	Small heat shock protein
0.95	0.36	2.80	orf19.5201.1	orf19.5201.1	Has domain(s) with predicted enzyme inhibitor activity
0.95	0.36	2.80	orf19.1064	ACS2	Acetyl-CoA synthetase
0.95	0.36	2.80	orf19.251	GLX3	Glutathione-independent glyoxalase
0.95	0.36	2.79	orf19.7585	INO1	Inositol-1-phosphate synthase
0.96	0.36	2.78	orf19.3590	IPP1	Putative inorganic pyrophosphatase
0.96	0.36	2.77	orf19.5466	RPS24	Predicted ribosomal protein
0.97	0.36	2.76	orf19.4135	PRC2	Putative carboxypeptidase
0.97	0.36	2.75	orf19.1409.1	orf19.1409.1	Ribosomal 60S subunit protein L22B

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Ratio H/Y normalized to biomass	Accession	Gene	Description
0.97	0.37	2.74	orf19.5645	MET15	O-acetylhomoserine O-acetylserine sulfhydrylase
0.98	0.37	2.73	orf19.542	HXK2	Hexokinase II
0.98	0.37	2.73	orf19.6745	TPI1	Triose-phosphate isomerase
0.98	0.37	2.72	orf19.953.1	COF1	Putative cofilin
0.98	0.37	2.71	orf19.3765	RAX2	Plasma membrane protein
0.98	0.37	2.71	orf19.734	GLK1	Putative glucokinase
0.98	0.37	2.71	orf19.4490	RPL17B	Ribosomal protein L17
0.99	0.37	2.70	orf19.4290	TRR1	Thioredoxin reductase
0.99	0.37	2.69	orf19.3997	ADH1	Alcohol dehydrogenase
0.99	0.37	2.69	orf19.2347	MNN2	Alpha-1,2-mannosyltransferase
1.00	0.37	2.67	orf19.3374	ECE1	Hypha-specific protein
1.01	0.38	2.64	orf19.3789	RPL24A	Predicted ribosomal protein
1.01	0.38	2.64	orf19.4755	KEX2	Subtilisin-like protease
1.02	0.38	2.62	orf19.2060	SOD5	Cu and Zn-containing superoxide dismutase
1.02	0.38	2.62	orf19.3324	TIF	Translation initiation factor
1.02	0.38	2.61	orf19.5968	RDI1	Putative rho GDP dissociation inhibitor
1.03	0.39	2.59	orf19.1789.1	LYS1	Saccharopine dehydrogenase
1.03	0.39	2.58	orf19.657	SAM2	S-adenosylmethionine synthetase
1.04	0.39	2.56	orf19.5996.1	RPS19A	Putative ribosomal protein S19
1.04	0.39	2.56	orf19.2864.1	RPL28	Putative ribosomal protein
1.04	0.39	2.56	orf19.5112	TKL1	Putative transketolase
1.05	0.39	2.55	orf19.6202	RBT4	Pry family protein
1.05	0.39	2.55	orf19.1665	MNT1	Alpha-1,2-mannosyl transferase
1.05	0.39	2.54	orf19.3294	MBF1	Putative transcriptional coactivator
1.05	0.39	2.53	orf19.789	PYC2	Putative pyruvate carboxylase
1.07	0.40	2.50	orf19.2841	PGM2	Ortholog of S. cerevisiae Pgm2
1.07	0.40	2.49	orf19.1153	GAD1	Putative glutamate decarboxylase
1.08	0.40	2.48	orf19.7611	TRX1	Thioredoxin
1.08	0.41	2.47	orf19.2452	orf19.2452	Protein of unknown function
1.08	0.41	2.46	orf19.437	GRS1	Putative tRNA-Gly synthetase
1.09	0.41	2.45	orf19.4393	CIT1	Citrate synthase
1.09	0.41	2.45	orf19.5964	ARF2	Putative ADP-ribosylation factor
1.09	0.41	2.44	orf19.4309	GRP2	Methylglyoxal reductase
1.10	0.41	2.42	orf19.6385	ACO1	Aconitase
1.11	0.42	2.39	orf19.7048.1	RPS28B	Putative ribosomal protein S28B
1.12	0.42	2.39	orf19.5830	LHS1	Protein similar to S. cerevisiae Hsp70p
1.12	0.42	2.38	orf19.3226	orf19.3226	In intracellular sterol transport and extracellular region
1.12	0.42	2.38	orf19.3014	BMH1	Sole 14-3-3 protein in C. albicans
1.13	0.42	2.37	orf19.4899	GCA1	Extracellular/plasma membrane-associated glucoamylase
1.13	0.42	2.36	orf19.2937	PMM1	Phosphomannomutase
1.13	0.42	2.35	orf19.4193.1	RPS13	Putative ribosomal protein of the small subunit
1.15	0.43	2.33	orf19.5741	ALS1	Cell-surface adhesin
1.15	0.43	2.32	orf19.2622	YPT31	Resistance to toxic ergosterol analog

Ratio Y/H per 100µg protein	Ratio Y/H normalized to biomass	Ratio H/Y normalized to biomass	Accession	Gene	Description
1.16	0.44	2.29	orf19.6906	ASC1	40S ribosomal subunit similar to G-beta
1.16	0.44	2.29	orf19.6327	HET1	Putative sphingolipid transfer protein
1.18	0.44	2.25	orf19.4336	RPS5	Ribosomal protein S5
1.19	0.45	2.23	orf19.4602	MDH1-1	Predicted malate dehydrogenase precursor
1.23	0.46	2.17	orf19.2677	orf19.2677	Putative GPI transamidase component
1.23	0.46	2.17	orf19.4152	CEF3	Translation elongation factor 3
1.24	0.46	2.16	orf19.2028	MXR1	Putative methionine sulfoxide reductase
1.24	0.47	2.15	orf19.4150	orf19.4150	Putative glutaredoxin
1.25	0.47	2.14	orf19.1946	orf19.1946	Aldose 1-epimerase-related protein
1.25	0.47	2.13	orf19.2769	orf19.2769	Putative protease B inhibitor
1.25	0.47	2.13	orf19.1671	UTR2	Putative GPI anchored cell wall glycosidase
1.26	0.47	2.11	orf19.7417	TSA1	TSA/alkyl hydroperoxide peroxidase C
1.27	0.48	2.10	orf19.6975	YST1	Ribosome-associated protein

Table S-3 MS data of SPE experiment

Accession	Description	ΣCoverage	Σ# Peptides	Σ# PSMs
orf19.3374	ECE1	88.56	54	256
	Sequence	# PSMs	Accessions	Modifications
	SIIGIIMGILGNIPQVIQIIMSIVKAFKGNKR	50	orf19.3374	
	SIETVGIENAAQIVSER	24	orf19.3374	
	DGVPDVGLNLVANAPR	22	orf19.3374	
	SIIGIIMGILGNIPQVIQIIMSIVKAFKGNKR	18	orf19.3374	M21(Oxidation)
	DGANDDVANAVVRLPEIVA	12	orf19.3374	
	DISSLIEEYFGKA	11	orf19.3374	
	VATGVQQSIENAK	10	orf19.3374	
	SAESALKDSQPVK	6	orf19.3374	
	RVATGVQQSIENAK	6	orf19.3374	
	NAAQIVSER	5	orf19.3374	
	DGANDDVANAVVRLPEIVAR	5	orf19.3374	
	LVANAPR	4	orf19.3374	
	TGVQQSIENAK	4	orf19.3374	
	AVDTAMTSVASTK	4	orf19.3374	M6(Oxidation)
	DGANDDVANAVVR	4	orf19.3374	
	DISSLIEEYFGK	4	orf19.3374	
	DGLEDFLDELLQRLPQ	4	orf19.3374	
	GVPDVGLNLVANAPR	4	orf19.3374	
	SAESALKD	3	orf19.3374	
	EDIDSVVAGIIADMPFVVR	3	orf19.3374	
	NVFDGVSETVQQAKR	3	orf19.3374	
	DGLEDFLDELLQRLPQL	3	orf19.3374	
	VATGVQQSIENAKR	2	orf19.3374	
	SIIGIIMGILGNIPQVIQIIMSIVKAFKGNK	2	orf19.3374	M21(Oxidation)

Accession	Description	ΣCoverage	Σ# Peptides	Σ# PSMs
	SIVKAFKGNK	2	orf19.3374	
	VGLNLVANAPR	2	orf19.3374	
	VVRLPEIVAR	2	orf19.3374	
	VPDVGLNLVANAPR	2	orf19.3374	
	ITRSAESALKDSQPV	2	orf19.3374	
	EDIDSVVAGIIADMPF	2	orf19.3374	M14(Oxidation)
	DGANDDVANAVVRLPEIV	2	orf19.3374	
	DVAPAAPAAPADQAPTVPAPQEFNTAITK	2	orf19.3374	
	DVAPAAPAAPADQAPTVPAPQEFNTAITKR	2	orf19.3374	
	DAGSVALSNLIKK	2	orf19.3374	
	EFNTAITKR	1	orf19.3374	
	SAESALKDSQPV	1	orf19.3374	
	SAESALKDSQPVKR	1	orf19.3374	
	AVDTAMTSVASTK	1	orf19.3374	
	GIENAAQIVSER	1	orf19.3374	
	RSAESALKDSQPV	1	orf19.3374	
	DGANDDVANAVVRL	1	orf19.3374	
	ETVGIENAAQIVSER	1	orf19.3374	
	DGLEDFLDELLQRL	1	orf19.3374	
	ANDDVANAVVRLPEIVA	1	orf19.3374	
	DGANDDVANAVVRLPEI	1	orf19.3374	
	DGVPDVGLNLVANAPRLIS	1	orf19.3374	
	EDIDSVVAGIIADMPFVVR	1	orf19.3374	M14(Oxidation)
	DGLEDFLDELLQRLPQLI	1	orf19.3374	
	DGLEDFLDELLQRLPQLIT	1	orf19.3374	
	DGANDDVANAVVRLPEIVARVA	1	orf19.3374	
	DGLEDFLDELLQRLPQLITR	1	orf19.3374	
	NKREDIDSVVAGIIADMPFVVRAVDTAMTSVASTKRDGANDDVANAVVRLPEIVA	1	orf19.3374	M17(Oxidation)M28(Oxidation)
	GIIADMPFVVR	1	orf19.3374	M6(Oxidation)
	RVATGVQQSIENA	1	orf19.3374	
	EDIDSVVAGIIADMPFV	1	orf19.3374	M14(Oxidation)
	LISNVFDGVSETVQQAKR	1	orf19.3374	
	STKRDGANDDVANAVVRLPEIVAR	1	orf19.3374	
orf19.6928	SAP9	5.88	3	23
	Sequence	# PSMs	Accessions	Modifications
	GESKDDLSPEDDSNPR	11	orf19.6928	
	RGESKDDLSPEDDSNPR	10	orf19.6928	
	NERSFGHGTGVKLNE	2	orf19.6928	
orf19.1327	RBT1	4.02	3	21
	Sequence	# PSMs	Accessions	Modifications
	EAEIANKDGTIEK	16	orf19.1327	
	ELDEFEELSNDGVTHS	3	orf19.1327	
	ELDEFEELSNDGVTH	2	orf19.1327	
orf19.3004	orf19.3004	12.44	3	6
	Sequence	# PSMs	Accessions	Modifications

Accession	Description	ΣCoverage	Σ# Peptides	Σ# PSMs
	KNVIDPASLKEGSAEEEQKDK	4	orf19.3004	
	KNVINLSNFIETPS	1	orf19.3004	
	KNLFDLTKFQQSGQPI	1	orf19.3004	
orf19.5542	SAP6	6.46	2	5
	Sequence	# PSMs	Accessions	Modifications
	SLVDPDDPTVESK	3	orf19.5542	
	GPVAVKLDNEIITY	2	orf19.5542	
orf19.1597	ABG1	6.25	1	4
	Sequence	# PSMs	Accessions	Modifications
	DKQSDNENDAEIEQEIER	4	orf19.1597	
orf19.2452	orf19.2452	3.13	1	4
	Sequence	# PSMs	Accessions	Modifications
	SSSDTTTSGTTIH	4	orf19.2452	
orf19.6741	orf19.6741	3.13	1	3
	Sequence	# PSMs	Accessions	Modifications
	DGVGSKMVSINPR	2	orf19.6741	
	DGVGSKMVSINPR	1	orf19.6741	M7(Oxidation)
orf19.3642	SUN41	2.63	1	2
	Sequence	# PSMs	Accessions	Modifications
	EDCDKTSFHGH	2	orf19.3642	
orf19.5585	SAP5	5.50	1	1
	Sequence	# PSMs	Accessions	Modifications
	LFLEFTPSEFPVDETGRDGDVDK	1	orf19.5585	
orf19.1442	PLB4.5	2.28	1	1
	Sequence	# PSMs	Accessions	Modifications
	DTQNNDEKEEFIGVVR	1	orf19.1442	
orf19.1426	orf19.1426	2.12	1	1
	Sequence	# PSMs	Accessions	Modifications
	PTFDLVYERTPTLEV	1	orf19.1426	
orf19.894	orf19.894	1.15	1	1
	Sequence	# PSMs	Accessions	Modifications
	ALLRVQSS	1	orf19.894	
orf19.1490	MSB2	1.06	1	1
	Sequence	# PSMs	Accessions	Modifications
	YQQENEITPADNIDK	1	orf19.1490	
orf19.3681	orf19.3681	1.03	1	1
	Sequence	# PSMs	Accessions	Modifications
	ADMVVDAID	1	orf19.3681	M3(Oxidation)
orf19.663	GIN4	0.89	1	1
	Sequence	# PSMs	Accessions	Modifications
	IRKFNTILPKHE	1	orf19.663	

Table S-4 Sample loading list of patients´sera

Addition of 1st. Ab.(dilution 1:1000 in blocking solution 0,5% casein in TBS, 100µl /well); incubate for 30min at RT

	1	2	3	4	5	6	7	8	9	10	11	12
A	A91	A105	E43	E44	E97	A	B	C	D	E	F	G
B	A91	A105	E43	E44	E97	A	B	C	D	E	F	G
C	H	I	J	P56	P083	P091	P112	P129	P279	P283	P289	P413
D	H	I	J	P56	P083	P091	P112	P129	P279	P283	P289	P413
E	P451	P1127	P1205	P1255	P1386	P2649	P2949	NC1	NC2	NC3	NC4	NC5
F	P451	P1127	P1205	P1255	P1386	P2649	P2949	NC1	NC2	NC3	NC4	NC5
G	NC6	NC7	NC8	NC9	NC10	G1	G2	G3	G4	G5	G6	G7
H	NC6	NC7	NC8	NC9	NC10	G1	G2	G3	G4	G5	G6	G7

Table S-5 TMT6plex sample labeling list

TMT-Ratio (Yeast/Hypha)	sample
127/126	2-yeast/1-Hypha
129/126	3-yeast/1-Hypha
131/126	4-yeast/1-Hypha
127/128	2-yeast/2-Hypha
129/128	3-yeast/2-Hypha
131/128	4-yeast/2-Hypha
127/130	2-yeast/3-Hypha
129/130	3-yeast/3-Hypha
131/130	4-yeast/3-Hypha

Abbreviation

2D	two dimensional
A.	*Aspergillus*
A.P.	acute pancreatitis
ACN	acetonitrile
AGC	automatic gain control
ALS	agglutinin-like sequence
amu	unified atomic mass unit, dalton
APC	antigen-presenting cell
ATP	adenosine triphosphate
BCR	B cell receptor
°C	celsius degree
C.	*Candida*
CGD	*Candida* genome database
CHAPS	3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate
cm	centimeter
CO_2	carbon dioxide
compl.	complement
COPD	chronic obstructive pulmonary disease
CWP	cell-wall protein
Cys	cystein
Da	dalton
DDA	data-dependent acquisition
DP	dolichol phosphate
DPGS	dolichol phosphate glucose synthase
DPMS	dolichol phosphate mannose synthase
DTT	dithiothreitol
EDTA	ethylenediaminetetraaacetic
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
ESI	electrospray ionization
et al.	and others
etc.	and so forth
Fas	fasciclin
FDR	false discovery rate
FWHM	full width at half maximum
g	gram
GE	gel electrophoresis
GO	gene ontology
GPI	glycosylphosphatidylinositol
h	hour
HCCA	α-cyano-4-hydroxycinnamic acid
HCD	Higher-energy collisional dissociation
HIV	human immunodeficiency virus

HRP	horseradish peroxidase
i.e.	that is
IC	invasive candidiasis
IgG	Immunoglobulin G
IPG	Immobilized pH gradient
k	kilo
K	lysine
kV	kilo volt
L	liter
LC	liquid chromatography
M	molecular mass marker proteins
m	meter
MALDI-TOF	matrix-assisted laser desorption/ionization-time of flight
Met	methionine
mg	milligram
MHC	major histocompatibility complex
min	minute
mM	millimolar
MOPS	3-(N-morpholino)propanesulfonic acid
MR	mannose receptor
MS	mass spectrometry
ms	millisecond
MWCO	molecular weight cut-off
NL	non-linear
nm	nanometer
OD	optical density
OP	operation
OST	oligosaccharyl transfer complex
PA	polyacrylamide
PAMP	pathogen-associated molecular pattern
PAS	periodic acid-schiff
PBS	phosphate buffered saline
PC	proprotein convertase
PES	polyethersulfone
Pir	proteins with internal repeats
PLGS	proteinlynx glolbal server
ppm	parts per million
PRR	pattern recognition receptor
PSM	peptide spectral match
PTM	post-translational modification
PVDF	polyvinylidene difluoride
R	arginine
RFP	red fluorescent protein
rpm	rotations per minute
RSLC	rapid separation liquid chromatography
SC	systemic candidasis

SDS	sodium dodecyl sulfate
SERPA	serological proteome analysis
SIRS	systemic inflammatory response syndrome
SPC	subilisin-like Proprotein Convertase
SPE	solid phase extract
Spp.	*species pluralis*
TBS	tris buffered saline
TCA	trichloroacetic acid
TCR	T-cell receptor
TFA	trifluoracetic acid
TFE	trifluoroethanol
TLR	toll-like receptor
TMB	tetramethylbenzidine
TMT	tandem mass tag
UPLC	ultra performance liquid chromatography
UV	ultraviolet
V	voltage
v/v	volume/volume
Vhs	voltage hours
W	watt
w/v	weight/volume
wt	wild type
YNB	yeast nitrogen base
YPD	yeast Nijmegen breakage syndrome
µL	microliter
µm	micrometre

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List of publications

Parts of this thesis are included in the following publications:

Research articles:

Ting Luo, Thomas Krüger,Uwe Knüpfer, Lydia Kasper, Natalie Wielsch, Bernhard Hube , Andreas Kortgen, Michael Bauer, Evangelos J. Giamarellos-Bourboulis, George Dimopoulos, Axel A. Brakhage, Olaf Kniemeyer (2016) "Immunoproteomic analysis of antibody responses to extracellular proteins of *Candida albicans* revealed the importance of glycosylation for antigen recognition" *J Proteome Res.* (in Revision)

David L. Moyes, Duncan Wilson, Jonathan P. Richardson, Selene Mogavero, Shirley X. Tang, Julia Wernecke, Sarah Höfs, Remi L. Gratacap, Jon Robbins, Manohursingh Runglall, Celia Murciano, Mariana Blagojevic, Selvam Thavaraj, Toni M. Förster, Betty Hebecker, Lydia Kasper, Gema Vizcay, Simona I. Iancu, Nessim Kichik, Antje Häder, Oliver Kurzai, **Ting Luo**, Thomas Krueger, Olaf Kniemeyer, Ernesto Cota, Oliver Bader, Robert T. Wheeler, Thomas Gutsmann, Bernhard Hube and Julian R. Naglik (2016) "Candidalysin: A fungal peptide toxin critical for mucosal infection", *Nature*, accepted

Reviews:

Thomas Krüger, **Ting Luo**, Hella Schmidt, Iordana Shopova and Olaf Kniemeyer (2015) "Challenges and Strategies for Proteome Analysis of the Interaction of Human Pathogenic Fungi with Host Immune Cells", *Proteomes*, *3*(4), 467-495

Parts of this thesis were presented at the following conferences:

Ting Luo, Ilse Jacobsen, Bernhard Hube, Axel A. Brakhage, Olaf Kniemeyer (2012) "Identification of protein antigens from *Candida albicans* for fungal sepsis diagnostics", the 18th International Society for Human and Animal Mycology (ISHAM 2012) in Berlin, Germany, Poster presentation

Ting Luo, Ilse Jacobsen, Bernhard Hube, Axel A. Brakhage, Olaf Kniemeyer (2012) "Identification of protein antigens from Candida albicans for fungal sepsis diagnostics", The DAAD Programme German-Chinese Symposium "Modern Applications of Biotechnology" and GCLB meeting in Dresden, Germany, Abstract

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2013)

"Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", Proteomic Forum 2013, in Berlin, Germany, Poster presentation

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2013) "Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", Weimar Sepsis Update 2013, in Weimar, Germany, Poster presentation

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Aleš Svatoš, Natalie Wielsch, Axel A. Brakhage, Olaf Kniemeyer (2013) "Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", 7th Central and Eastern European Proteomics Conference (CEEPC), in Jena, Germany, Poster presentation

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2015) "Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", Sixth FEBS advanced lecture course Human Fungal Pathogens, La Colle sur Loup, France, Poster presentation

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2015) "Searching for diagnostic biomarkers for Candidemia", Statusworkshop " Eukaryotische Krankheitserreger" der Deutsche Gesellschaft für Hygiene und Mikrobiologie (DGHM), in Erlangen, Germany, Talk

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2015) "Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", 27th Annual Conference of Gemeinschaft Chinesischer Chemiker und Chemieingenieure in Deutschland (GCCCD), in Jena, Germany, Abstract

Ting Luo, Ilse Jacobsen, Bernhard Hube, Michael Bauer, Andreas Kortgen, Evangelos J.Giamarellos-Bourboulis, Axel A. Brakhage, Olaf Kniemeyer (2015) "Identification of antigens from the secretome of *Candida albicans* for fungal sepsis diagnostics", 49. Wissenschaftliche Tagung der Deutschsprachigen Mykologischen Gesellschaft e.V. und 1st International Symposium of the CRC/Transregio FungiNet/ DMykG 2015, in Jena, Germany, Talk

Teaching activities:

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Ehrenwörtliche Erklärung

Die geltende Promotionsordnung der Biologisch-Pharmazeutischen Fakultät der Friedrich-Schiller-Universität Jena ist mir bekannt. Die vorliegende Dissertation habe ich selbständig verfasst und keine anderen als die von mir angegebenen Quellen, persönliche Mitteilungen und Hilfsmittel benutzt.

Bei der Auswahl und Auswertung des Materials haben mich die in der Danksagung meiner Dissertation genannten Personen unterstützt. Personen, die bei der Anfertigung der Publikationen beteiligt waren, sind in der Publikationsliste angegeben.

Ich habe die Dissertation noch nicht als Prüfungsarbeit für eine staatliche oder andere wissenschaftliche Prüfung eingereicht. Ferner habe ich nicht versucht, diese Arbeit oder eine in wesentlichen Teilen ähnliche oder eine andere Abhandlung bei einer anderen Hochschule als Dissertation einzureichen.

Jena, _____

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Ting Luo